Vol. XII, No. 2 May, 1925

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF ECONOMIC BIOLOGISTS

BY

W. B. BRIERLEY

AND

D. WARD CUTLER

CAMBRIDGE UNIVERSITY PRESS
LONDON: FETTER LANE, E.C. 4
also
H. K. LEWIS & CO., LTD., 136, GOWER STREET, LONDON, W.C. 1
WHELDON & WESLEY, LTD., 2, 3, 4, ARTHUR ST., NEW OXFORD ST., W.C. 2
PARIS: LIBRAIRIE HACHETTE & CIE.
CHICAGO: THE UNIVERSITY OF CHICAGO PRESS
(AGENTS FOR THE UNITED STATES)
BOMBAY, CALCUTTA, MADRAS: MACMILLAN & CO., LTD.
TOKYO: THE MARUZEN-KABUSHIKI-KAISHA

Price Twelve Shillings net

PRINTED IN GREAT BRITAIN

The Association of Economic Biologists

CONTENTS OF VOL. XII, No. 2

VOLUME XII MAY, 1925 No. 2

THE DOWNY MILDEW OF THE HOP AND ITS EPIDEMIC OCCURRENCE IN 1924

BY E. S. SALMON AND W. M. WARE.

(*Mycological Department, South Eastern Agricultural College, Wye, Kent.*)

(With Plates VII–IX and 2 Text-figures.)

CONTENTS.

1. HISTORICAL.

THE Downy Mildew of the Hop was first recorded(19) in 1905, in Japan, by Professor Kingo Miyabe and Mr Y. Takahashi. The fungus was described as a new species, under the name of *Peronoplasmopara Humuli*. It was first noticed on cultivated hops in two places, viz. "on the leaves of the cultivated hop-vines in the experimental plat of the Hokkaido Agricultural Experiment Station in Sapporo, and in the hop-field belonging to the Sapporo Brewery Company (Sapporo)." The following extract relates to the incidence of the disease in the latter locality: "On June 15th...we found the mildew to have already begun to spread to an alarming extent throughout the field. A portion of the field adjoining the place where the hop-vines were collected and burnt the previous autumn was very badly attacked. The lower leaves of the vine were at that time most infected, but the disease had already spread to some of the upper leaves. Judging from the extent to which the fungus had spread in the field, we may safely infer that the disease had existed there for many years without drawing attention. Messrs S. Fujita and

J. Kasahara of the Company, struck with the seriousness of the case, at once took active measures to combat the disease. By thoroughly spraying with the Bordeaux mixture and by systematic picking of the affected leaves, they were able to prevent the spread of the disease for the rest of the year. The fungus in question seems to be peculiar to Japan, as there are no records of the occurrence of the downy mildew on the hop-vines either in Europe or America, for such a destructive parasite on such an important crop is scarcely likely to have passed unnoticed there."

During 1905 Mr J. Hanzawa and Prof. G. Yamada, two Japanese mycologists, found the same fungus on the native wild hop, *Humulus Lupulus* L. var. *cordifolius* Maxim., in other parts of Japan.

Professor Miyabe and Mr Takahashi remark: "These facts prove beyond doubt that the mildew fungus is indigenous to this country growing on the wild hop-vine and has recently found a more congenial host in the cultivated hop-vines introduced from America and Europe."

In a letter received from Prof. Miyabe in 1916, he stated that he had found *P. Humuli* on *Humulus japonicus* also, adding, "This fungus attacks the fruits (cones) of the cultivated Hop, making them quite worthless."

In 1909 Dr J. J. Davis reported (9) the occurrence of a Downy Mildew on the hop in North America: "A downy mildew was observed on *Humulus Lupulus* in Wisconsin in 1909 which is referred to *Pseudoperonospora celtidis* (Waite) Wilson as var. *Humuli* n. var." In 1914, Prof. G. W. Wilson, in his "Studies in North American *Peronosporales*" (26), named the American fungus *Pseudoperonospora Humuli* (Miyabe and Takahashi), stating: "Some years later [than the discovery of the fungus in Japan] Dr Davis collected a fungus on the wild hops of Wisconsin which he considered quite close to *P. Celtidis*, but entitled to subspecific rank. Through the kindness of Mrs Flora W. Patterson the writer has been able to examine Japanese material of this species and to compare it with specimens submitted by Dr Davis. As in the case of *Kawakamia Cyperi* the measurements of the American material do not agree exactly with those of the Japanese specimens but otherwise the similarity is too great to admit a question of their identity."

In a letter, together with specimens of the fungus, which we received from Dr Davis in 1921, he states: "I found the Downy Hop Mildew again last summer and do not doubt that it is indigenous[1]."

[1] In the *Review of Applied Mycology*, I, 73 (1922), in a review of Mrs N. L. Alcocks' article "Protection against Fungi from Abroad" (*Journ. Min. Agric.* XXVIII, 455 (1921))

According to information kindly supplied in October 1924 by Dr W. W. Stockberger, of the U.S. Department of Agriculture, Dr Davis has written as follows (*Trans. Wis. Acad. Sci. Arts and Letters*, 192, 671): "In the first number of these notes there was mention of the occurrence of *Plasmopara humuli* Miyabe and Takahashi[1] on *Humulus Lupulus* at Racine in South-Eastern Wisconsin. This seems to be the first, and as yet the only, American locality from which this Japanese mildew has been reported. In September, 1915, it was collected on the same host near Lynxville on the Mississippi and at Gays Mills and Petersburg in the Kickapoo Valley in Western Wisconsin. At Lynxville the Japanese host *Humulus japonicus* was abundant on vacant lots and it was also observed at Gays Mills in cultivation and as an escape, but the mildew was not found on this species." We are indebted to Miss E. M. Wakefield for the following extract from a more recent paper written by Dr Davis[2]: "*Plasmopara humuli* Miy. and Takah.—In south-eastern and south-western Wisconsin. A scanty development has also been seen in central Wisconsin. It appears to be indigenous. It was collected at Caryville in the Chippewa valley in July, 1920, with oospores."

Information was sought from the U.S. Department of Agriculture as to whether the Downy Mildew had appeared in the commercial hop-yards in America and if so what economic damage it was causing. Dr Stockberger has communicated the following information: "So far as I am informed this fungus has not attacked our hop-plants to the extent that noticeable injury has resulted" (Oct. 1924); and "This disease appears to be extremely rare in this country and almost never to be found in our cultivated fields of Hops" (Dec. 1924).

In Europe the fungus was first found (21), in 1920, in the Experimental Hop-garden and Nursery, at Wye College, Kent. The circumstances attendant on this outbreak, the nature of the disease caused and its spread at Wye in the following years have a special importance, for any light they may throw on the question, discussed at the end of this paper, of the origin in this country of a new disease of cultivated hops. The fungus was first found at Wye in 1920 on only a few plants, viz. on some seedlings raised from seed of the truly wild hop

the following sentence occurs: "Downy mildew of hops (*Peronoplasmopara humuli*) was imported from Japan into the United States and is there spreading." This statement as to the importation of the fungus is a *lapsus calami*, as reference to the original article shows.

[1] The species was referred to this genus by Saccardo and Trotter in *Sacc. Syll. Fung.* XXI, 861 (1912).

[2] "Notes on parasitic fungi in Wisconsin. IX. *Trans. Wis. Acad. Sci.* XXI (1924).

(*H. Lupulus*) obtained in 1913 from Italy. These plants were so late-flowering that their hops were unfit to pick when those of the surrounding plants were gathered in September, and it was not until October that the plants in question were examined. The examination was very thorough, as the susceptibility or resistance of each plant to the attacks of *Sphaerotheca Humuli* was being tabulated[1]. It was then noticed that the leaves of some plants were spotted in an unusual manner, the characteristic feature of the spots being their angular outline. Only a few spots were observed on any one leaf, and the patches of conidial growth of the downy mildew on them was slight. As a disease it was insignificant in appearance, causing no appreciable harm. While it cannot be stated that the fungus was not present in 1920 on other hop-plants in the Experimental Hop-garden, it appears almost certain that it was in this year that the inception of the disease took place[2]. The season 1921 was very dry, and although a search was made for the fungus on the same plants on which it had been observed in 1920, as well as elsewhere in the Experimental garden, it could not be found. Early in September 1922—a very wet season—the fungus reappeared, showing increased virulence and attacking not only the leaves of a large number of plants, but also in a few instances the hop-cones. The plants attacked were of the most varied origin, *e.g.* seedlings of the wild hop from Italy; hybrid seedlings raised from German and American varieties (including the var. *neo-mexicanus*) crossed with various male hops. In October of the same year oospores of the fungus were found in affected leaves. Sufficient knowledge of the fungus had by then been obtained to enable it to be identified as the Downy Mildew recorded in Japan and called *Peronoplasmopara Humuli*.

Influenced by the fact that both seeds and plants of *H. Lupulus* var. *cordifolius* had been imported (in 1917) from Japan, and that these seedlings and plants had been planted in the Experimental Hop-garden, and that on several occasions from 1908 onwards roots of American varieties of hops (including var. *neo-mexicanus*) had been obtained from the United States, the opinion was expressed(21) at the time that the

[1] See E. S. Salmon, "On Forms of the Hop resistant to Mildew, V." *Annals of Applied Biology*, VIII. 146 (1921); and E. S. Salmon and H. Wormald, "A Study of the Variation in Seedlings of the Wild Hop." *Journ. of Genetics*, XI, 243 (1921).

[2] The Superintendent of the British Rainfall Organization of the Meteorological Office has kindly supplied us with the figures of the monthly rainfall from May to October, 1920, at the two recording stations (Chilham and Kennington) nearest to Wye. In the months of May, June, August and October, the rainfall was slightly below the normal; in July and September it was considerably (1·94 in. and 1·01 in.) above.

fungus had probably been introduced into this country from either Japan or America[1]. The fresh facts that came to light during 1924 (see below) make the attribution of the disease to a foreign source very doubtful.

In 1923 a new form of the disease was observed, not hitherto reported (we believe) from either Japan or America. Under the attack of the mildew, more or less hypertrophied, spike-like shoots of the hop develop, on the stem and leaves of which conidiophores are produced in great abundance. These are figured in Plates VIII and IX, and are described more fully below. Except for its appearance in this new and alarming form, the disease was not noticeably worse in 1923 than in 1922; a considerable number of plants showed the fungus on their leaves, but in no instance was it found on the hop-cones. It must be noted here that 1923 was a dry season. In 1924—a record wet season—the disease showed itself from one end of the hop-garden to the other ($3\frac{1}{2}$ acres) attacking the leaves and cones to a greater extent than it had ever done before, and appearing in the spike form on some hundreds of plants.

Before passing on to record the spread of the disease to other parts of Kent and the epidemic appearance of a Downy Mildew in hop-gardens and hop-nurseries generally and on wild hops, all of which took place during 1924, it will be well first to describe more closely the fungus and the disease it produces on cultivated hops. This information is based on observations made at Wye during the past five years.

2. Effect of the disease on the host-plant and economic damage caused.

The general appearance of the Downy Mildew on the Hop is as follows. On the leaf the fungus causes characteristic angular spots, dark brown above and paler on the lower surface of the leaf. On the lower surface of these spots tufts of conidiophores appear; when the spots coalesce to form large patches, a conspicuous more or less matted growth of conidiophores is produced, which to the naked eye is blackish-grey in colour, or sometimes tinged with violet. The fungus is always confined to the lower surface of the leaf, and is most frequent by the side of the midrib, or occupying little islands of tissue bounded by the

[1] An Order ("The Destructive Insects and Pests Order of 1922") issued by the Ministry of Agriculture in May, 1922, declared it illegal to import any plant bearing the "Downy Mildew of Hops (*Peronoplasmopara Humuli* Miy. et Taka.)." Although the same Order gave the Ministry powers to prescribe special measures against the disease, it has not up to the present been considered advisable to take such action.

smaller veins, exactly as observers have recorded[1] with respect to the attacks made by *Rhysotheca viticola* (*Plasmopara viticola*) on the leaf of the Vine. On large hop-leaves the mildew sometimes occurs in more or less continuous patches extending along the margin, which as a result may turn brown and to some extent shrivel up. In addition to attacking the leaf, the disease may damage the hop-cone (strobile). The bracteoles are usually attacked first, and turn brown, so that the hop assumes a striped appearance, the vertical rows of brown, withering bracteoles alternating with bracts which are still green[2]. The conidiophores of the mildew are found in scattered, dark-coloured groups over the whole of the under surface of the bracteole.

In 1923 the disease appeared in a new form on many plants in the Experimental Hop-garden. In the spring and summer there were produced from the rootstocks in addition to normal, thin, climbing shoots, one or more shoots of a spike-like form, non-climbing and thickened, from a few inches to a foot or more in length, and bearing abnormally small, curled, brittle leaves, arising close together. On the leaves of such shoots, as well as on the surface of the stem, the mildew later produced its conidiophores in exceptionally dense masses, often blackening the entire lower surface of the leaf. Besides these basal spike-like shoots arising from the ground, similar diseased growths occurred terminating otherwise apparently healthy stems when they had reached lengths varying from 5 feet (at the "breast wire," when hops are grown on the wire-work system) up to, in a few cases, the final height at the top wire of 12 or 14 feet from the ground. (See Pl. VIII, fig. 3.) Frequently, also, similar small, diseased *lateral* shoots, a few inches long, appeared (at varying heights from the ground) on the otherwise normal and healthy main stem. (See Pl. VIII, fig. 4.)

The basal, spike-like shoots have also been observed on two- and three-year-old seedling hop plants. Pl. IX, fig. 6 shows a normal shoot produced by a seedling hop plant, and Pl. IX, fig. 5 a diseased shoot.

The development of the fungus in the basal spike-like shoots has not yet been followed, but the presence of a mycelium in the inner tissues of the stem and the fact that conidiophores may emerge from blister-like places on the surface of the stem suggest the possibility that the

[1] See *e.g.* G. de Istvánffi and G. Pálinkás in *Ann. l'Instit. Cent. Ampel. R. Hongrois*, IV, Pl. I, Figs. 2 and 3 (1913). Waite (*Journ. of Mycology*, VII, 105 (1892)) describes *Peronospora Celtidis* as forming on the host-leaf "definite angular spots, limited by the veinlets."

[2] A photograph of hop-cones attacked by the Downy Mildew was reproduced in the *Journal of the Ministry of Agriculture*, XXX, 431, Fig. 2 (1923).

mycelium may hibernate in the rootstock of the hop-plant or in its buds, and the following spring grow up inside the stem of the young shoot, distorting it as described above and ultimately producing conidiophores on the leaves[1]. If this explanation is rejected we must suppose that a mass-infection by germinating oospores takes place in the spring, bringing about the hypertrophy of the shoot. It is difficult to believe, however, considering the great vigour shown by the young basal shoots of the hop in spring, that such external infection could bring about the cessation of growth characteristic of these spike-like shoots. A similar mass-infection by conidia during the summer must be supposed to lead to the formation of the spike-like terminal shoots far above the ground, as well as that of the distorted lateral shoots[2], since it is difficult to imagine that the mycelium hibernating in the rootstock could travel up the stem such a distance without interfering with the normal growth.

As to the actual damage caused in the hop-garden at Wye, from the economic point of view, the injury to the hop leaves may perhaps be regarded as negligible. It is the fact that the hop-cone is liable to be attacked which makes the present disease of so great economic importance. In 1922 the hop-cones of only a few plants were affected, and the number of cones rendered unfit to pick was small; in 1923 the cones on a larger number of plants were attacked and in a few cases from quarter to half the crop was destroyed; in 1924 several hundreds of plants showed the disease in the cones, and a considerable number of these had the entire crop, or three-quarters of it, ruined by the disease. When it is remembered that the present cost of cultivating a hop-garden is over £100 per acre, and that any discoloration of the hop-cone fatally affects the market-price, the consternation that is felt by the farmer when a totally new disease such as the present appears, can be understood. The economic damage caused by the hypertrophy of the shoots, so far as it has been observed up to the present, has been negligible.

[1] Kühn (*Bot. Zeitung.* xxxi. 499 (1873)) and other observers have stated that a hibernating mycelium exists in the case of *Peronospora Schachtii* on Beet. A figure given by Dr Peters (*Deutsche Landwirtsch. Presse*, L, No. 13, p. 117, Fig. 63) illustrates a diseased, shortened shoot, with small curled leaves, arising in the second season from a Beet infected during the previous year; the characters shown appear similar in many respects to those of the hop-shoot reproduced in our Pl. IX, fig. 5. Dr P. A. Murphy has recently shown (*Nature*, cviii, Nov. 3, 1921) that a perennial mycelium exists in *Peronospora Schleideni* Unger, and that the new shoots produced in the second season from diseased bulbs are infected *ab initio*.

[2] When severely attacked by the conidia of *Sphaerotheca Humuli*, the small lateral shoots and also the inflorescences may be arrested in growth and transformed into knob-like structures.

Should it be proved, however, that the basal spike-like shoots are due to a perennial mycelium in the rootstock, a great danger would lie in the fact that year after year, early in the season, masses of conidia would be dispersed from such shoots. It must be pointed out that the above damage to the crop took place notwithstanding that during 1923 and 1924 certain measures were taken against the disease. In 1923 all spike-like shoots coming up from the rootstock were cut off, as soon as noticed, from below the soil level; and throughout the summer, as soon as the mildew was noticed on the leaves of any hop-plant, all its leaves, whether infected or not, were removed to a height of 5 or 6 feet, and burnt; the same treatment was given to the next plant on either side, for fear that it might have become infected. As soon as the hops had been picked in September, all the "bines" (stems) were cut down, and together with the leaves, carted off and burnt. In 1924 the process was commenced earlier; as soon as the mildew was found in May in one part of the garden, the lower leaves of all the plants throughout the whole hop-garden were removed up to a height of $1\frac{1}{2}$ feet from the ground, and again in a second operation, a few weeks later, to a height of 5 or 6 feet from the ground. A continued search was made throughout the growing season for spike-like shoots, which, whenever found, were removed; if several spikes came from one plant, this was grubbed up and burnt. It may be noted here that the removal of the basal spike-like shoots in 1923 did not, in many cases, prevent their reappearance in the same plant in 1924. In 1924 no less than 425 plants, out of the 6250 plants growing in the Experimental Hop-garden and Nursery, *i.e.* 6·8 per cent., developed basal spike-like shoots producing the Downy Mildew on their leaves. The plants thus attacked were of the most diverse origin and included the following: the truly wild *Humulus Lupulus* (grown from seed obtained from Italy in 1913); the wild hop from America, *H. americanus* var. *neo-mexicanus* (obtained in 1915 from Colorado); the wild hop from Japan[1], *H. Lupulus* var. *cordifolius* (grown from seed obtained from Japan in 1916); a number of German cultivated varieties (Lower Bavarian[2], Elsass, Aischgründer); American cultivated varieties (Oregon English Cluster and male plants from Oregon); seedlings of a "wild hop" from near Sidmouth, Devonshire (1916). The great majority of the

[1] The first time the Downy Mildew was observed on this plant was in August 1923, on the leaves of small and inconspicuous tufted diseased shoots arising laterally from the lower region of the stem, which was otherwise normal and healthy.

[2] An individual of this variety was very severely attacked in 1923, and produced during the summer large terminal "spikes" several inches long, on several of the "bines" (stems) at a height of 12 to 14 feet. All the diseased bines were removed as soon as noticed. In 1924 the plant produced both terminal and basal spike-like shoots.

plants, which produced "spikes" in 1924, however, were hybrid seedlings, the parents being the wild hop from Europe or America, including the var. *neo-mexicanus*, or cultivated European or American varieties. All the plants noted above had been grown at Wye for some years previous to the occurrence of the mildew.

The varieties which have proved most susceptible to the mildew, particularly as regards infection of the hop-cone, are the American variety *neo-mexicanus* and a plant (probably the wild hop of America (*H. americanus*)) obtained in 1916 from Manitoba, and hybrids raised from the above. The spike-like shoots were not noticed generally until 1923, but observations and a sketch made in a note-book in 1921 show that diseased lateral shoots occurred that year on certain plants (viz. on individuals of var. *neo-mexicanus* and on one hybrid plant[1]) although their connection with the Downy Mildew was not recognised at the time.

When the disease was noticed in 1922 as occurring on the leaves of a number of plants of different origin in the hop-garden (see above, p. 124), the reference numbers of some of the affected plants were noted. Of 27 plants then affected, one plant (or 3·7 per cent.) had shown a spike-like shoot by 1924. It is clear, therefore, that infection of the leaf (at any rate if this takes place in the autumn) does not necessarily lead to the formation of diseased shoots.

With regard to the weather conditions under which the disease spreads in the hop-garden, it is clear that wet weather favours the attack by the mildew on the hop-cones. In continued wet weather a crop of green, nearly ripe cones may be turned brown in a few days. On the other hand—a fact which is more surprising—the fungus can continue to produce conidiophores and conidia even in the hottest summer weather, as was observed during July and August, 1923.

3. Epidemic occurrence in England in 1924.

Previous to 1924, the Downy Mildew of the Hop had been recorded from one place only in Europe, viz. in the Experimental Hop-garden and Nursery, at Wye College, Kent.

In the summer of 1924 the fungus was found at the Fruit and Hop Research Station, East Malling, Kent. It was first found there on some plants of certain new varieties of hops, the cuttings of which had been received from Wye during the winter of 1922–23, and had been grown at

[1] This plant has remained free from lateral (and basal) "spikes" during the seasons 1922–24 inclusive.

East Malling in a nursery-bed during the season of 1923, and planted out in a row early in 1924. The few plants first found affected were at once destroyed, but later in the summer more plants of the same origin were found to be diseased and by August the fungus was also discovered on hundreds of hop plants in a nursery-bed near by. In each case all the plants, both affected and healthy, were promptly destroyed. The disease then appeared, on both the leaves and hop-cones, in the hop-garden at the Research Station, where new varieties of hops are being tested. During the wet weather of September, the disease increased rapidly day by day, and in some cases rows of 30 plants were more or less affected from one end to the other. The bines of those plants of which the cones were most severely affected were cut down and burned. These hop-cones had been ruined by the disease.

Believing at that time—for the reasons given above—that the Downy Mildew had been introduced into this country on imported plants—for which one of the present writers was responsible—it was imperative that all steps should be taken to stamp out the disease if that were possible. In the same nursery-bed at the Research Station, East Malling, where the cuts (from Wye College) were grown during 1923, other cuttings had been raised which had been distributed to hop growers during the winter of 1923–24. It became necessary to visit all the farms where these hops had been planted and inspect them. Consequently 20 farms in Kent, two in Sussex and one each in Hampshire, Worcester and Herefordshire were visited. The disease was found, on the plants sent out from East Malling Research Station, in the following places: Kent, 16 farms; Sussex, three farms. On the remaining farms in Kent, and on those in Hampshire, Worcestershire and Herefordshire the disease was not found. In some cases the hops had been planted out in a commercial hop-garden, and here the fungus was found on plants adjoining those obtained from East Malling Research Station. Thus, at Horsmonden, the Downy Mildew was found in a Fuggles hop-garden, and at Paddock Wood, in a Tutsham hop-garden. In some instances cuttings (and not rooted plants) had been sent out, and these had been planted by the farmer in a nursery, adjacent to cuttings saved from his own stock; the Downy Mildew was apparent on both sets of plants. Looking back with the knowledge we now possess with regard to a possible new source of the disease (which is mentioned below), it seems probable that whilst in many cases the disease on the farms mentioned was due to its introduction on plants from East Malling Research Station, in some of the cases, particularly where cuttings were grown, and where nettles or

wild hops occurred in the immediate vicinity, the infection proceeded from this other source.

In our anxiety to stop the spread of a new disease of the hop, the farmers concerned were persuaded to destroy not only all the hop plants obtained from East Malling but also in some cases rows of commercial varieties that were, or might be, affected, as well as some thousands of nursery plants which were or might have been contaminated.

The destruction of these hop plants was carried out under the impression that a Downy Mildew was present which had been introduced from abroad but which was otherwise unknown in this country. Although this view has disappeared before a wider knowledge, it may be pointed out here that complete destruction of the affected plants was the safest procedure because of the possibility—discussed below—that forms of the fungus showing different degrees of pathogenicity may exist.

During the end of September and throughout October, facts were discovered which threw an entirely new light on the subject of the recent outbreak of a Downy Mildew on the Hop in this country. At the end of September a Downy Mildew was found by one of us on the leaves of a "wild" hop[1] in a hedge by the roadside at Westwell, near Ashford, Kent. This discovery led to a general search being made on "wild" hops, and in the neighbourhood of Westwell the Downy Mildew was found in three other localities. The search continued, and during the early part of October the Downy Mildew was found on "wild" hops in the hedges in the following parishes in Kent: East Peckham, Paddock Wood, Wateringbury, Selling, Hastingleigh, Hothfield, Addington, between Pluckley and Egerton, and between Hothfield and Ashford. Mr J. Amos, Foreman Recorder at the Research Station, East Malling, also sent us the fungus on "wild" hops, growing on the railway bank near East Malling Halt. Some of the localities mentioned above were so far distant from Wye as to make it highly improbable that the Downy Mildew on these "wild" hops in the hedges could have been derived from the Experimental Hop-garden at Wye, but as all the above records were in the hop-growing county of Kent, it was desirable to ascertain whether the Downy Mildew occurred on hops at a distance from commercial hop-gardens. One of us visited the county of Middlesex, and after a short search found, on a waste piece of ground at Twickenham, "wild" hops with the Downy Mildew on their leaves. In two cases (one of which was at Twickenham) the Downy Mildew was observed on the

[1] In the south of England such hop-plants probably originate from cuttings of cultivated Hops.

hop-cones as well as on the leaves. Further confirmation was obtained as follows. One of the writers was aware of the existence of a wild hop growing in a hedge at Bickington, N. Devon, which had attracted attention as being an uncommon plant in the neighbourhood. Leaves from this plant were sent by a correspondent, and on these leaves the Downy Mildew occurred. Thus in two counties where hops are not cultivated, the Downy Mildew was found on the first hops examined.

It can be regarded as proved, then, that during the autumn of 1924 it was quite a common occurrence for "wild" hops to bear a Downy Mildew on the leaves and sometimes on the hop-cones. This fact is surprising because neither in mycological literature nor in the National Herbaria at Kew and at South Kensington does any record exist of a Downy Mildew occurring on the Hop. A Downy Mildew of the Hop is certainly a fungus endemic to this country (22). The generic and specific characters are described below.

Aware of the widespread occurrence during October of the Downy Mildew in the hedges in Kent, we began to search for it in that county in commercial hop-gardens generally. It was quickly found almost everywhere. At that time of the year (October) the hops were all picked, and the stems ("bines") still bearing leaves were either on the ground or hung over the "breast-wires" five feet from the ground. On the youngest of these leaves (more rarely on the older leaves) small patches of the Downy Mildew could be found. In a few cases the mildew was so abundant that the under surface of the leaf was conspicuously blackened by the dense, sooty-violet patches. As a general rule, however, the patches were quite small and inconspicuous, and bore only a few tufts of conidiophores. Time permitted of the search being made in only a limited number of hop-gardens, but it can be stated that in those of the district between Paddock Wood and Maidstone a general infestation occurred of the nature described above. Hop-gardens in the following parishes were found to be thus affected with a Downy Mildew: Paddock Wood, East Peckham, Wateringbury, Boughton Aluph, Wye, Selling.

On one farm at Paddock Wood the Downy Mildew was found on the leaves among the hop-cones. In this case it was reported to us by the farmer that some disease, of a nature unknown to him, had attacked the hop-cones and rapidly turned them brown just before they were fit to pick, thus ruining the crop and making it unfit to pick. The farmer cut the bines down, over some acres, in order to prevent the disease spreading. Although no Downy Mildew was found by us on the hops of these cut down dead plants when we visited the farm in October, it is

possible that this was a case where the Downy Mildew had attacked the crop[1].

Visits were then paid to farms where nursery stocks of commercial varieties of hops are raised in beds of thousands of sets and sold by the farmers. Nurseries were inspected at Malling, Boughton Aluph, Wye and Selling, and the plants therein were found to be heavily infested with a Downy Mildew. Thus at Boughton Aluph practically all the plants ("rooted sets") of a Golding variety bore patches of the mildew; at Wye and Selling the plants of Bramling, Tutshams, etc. were similarly infected. In the case of some of the nurseries these nursery plants were already sold and will have been distributed during the winter of 1924–25 to hop-growers not only in Kent but also in Surrey, Hampshire, Worcestershire and Herefordshire. In the case of one nursery, the plants of which were already sold to various growers in the above noted counties, it is not too much to say that every plant was more or less affected with the Downy Mildew. There is the probability therefore that every batch of plants sent out from this nursery carried, in the soil attached to the roots, fragments of leaves containing oospores, or free oospores. The possibility also exists that the fungus may be transported in the form of mycelium in the stem. Leaves from nursery plants were also obtained from various farms in the Weald of Kent and also from Herefordshire[2] and in each case a Downy Mildew was found.

On the greater number of farms mentioned above, where the Downy Mildew was found in the hop-garden or in the nursery, no material from Wye or East Malling Research Station had been planted, and the origin of the disease was therefore certainly to be sought elsewhere. Circumstantial evidence gradually led us to consider the possibility of the Downy Mildew of the Nettle, *Peronospora Urticae* (Lib.) de Bary of mycological text-books, being concerned. During the wet season of 1924 this species was practically ubiquitous in Kent, chiefly on *Urtica dioica*, but also commonly on *U. urens*. It was by no means an un-

[1] Caution is needed in attributing the cause to the Downy Mildew, because in 1924 in England, as in other hop-growing countries, a disease, apparently of a physiological nature and not due to any organism, appeared in many parts of Kent on the ripening hop-cones and discoloured them. It seems probable that it was caused by the persistent cold, wet weather. Descriptions of this disease, known variously in Belgium, Germany and Bohemia as "la maladie nouvelle," "la maladie des houblons de 1924" and "maladie rouge," and considered by scientists on the Continent to be due to the unfavourable weather conditions, will be found in *Le Petit Journal du Brasseur* (Bruxelles), xxxii, Sept. 19 and 26, Oct. 10 (1924).

[2] We are indebted to Messrs Wimshurst, of Tibbs Court, Brenchley, and to Major Croft, of Bartestree, Hereford, for kindly obtaining these specimens.

common occurrence to find diseased nettles in the closest proximity to hops, both in hop-nurseries and in roadside hedges, with the stem of the hop-plant twining round the nettle. In one case it was only where this occurred that the hop-plant (in the hedge) was affected with Downy Mildew, the others near by being free. In many hop-gardens, too, in which we found diseased hops the hedges bordering them contained nettles affected with the Downy Mildew.

Before passing on to a comparative study of the Downy Mildew of the Hop and of the Nettle, it will be well to mention here that during the autumn of 1924 the occurrence of "a *Peronospora*" on cultivated hops in Württemberg, Germany, was reported to us in a letter (Dec. 1924) by Dr Lang, of the Württembergische Landes-Anstalt für Pflanzenschutz, Hohenheim. No details of this outbreak were given to us nor have they been published up to the present. It is obvious, however, that the circumstances attending this sudden appearance of a Hop Downy Mildew in another country in Europe, totally unconnected with the first, must be taken into account, and may be expected to throw light on the problem of how this new disease of the Hop arose.

4. A comparative study of the Downy Mildew of the Hop and of the Nettle.

The microscopical details of the fungus on the Hop are as follows. The conidiophores show great variation in height, a character probably affected by age, density of growth, vigour of host and by weather conditions. Only a few measurements, thought to be typical, were therefore made. The main stem is swollen at the base and measurements of height, made from the top of this swelling to the tip of the highest branch, were found to vary between 190μ and 430μ[1]. The conidiophore is aseptate, colourless and 4–8μ thick; the branching (which may be three or four times repeated) is sometimes almost strictly dichotomous, but is variable and at the other end of the series approaches the monopodial type; the ultimate branches are thin, straight or slightly recurved, and under low magnification appear pointed. (See p. 136, Fig. 1.) Usually the tendency to dichotomy is shown by some, at any rate, of the branches. The conidia arise as small swellings at the tips of the branches of a young conidiophore and eventually assume an oval shape. At the point of attachment there is a clear spot in the wall and at the opposite end a small papilla or apiculus is developed. When the conidium becomes detached,

[1] All the conidiophores measured were mature, having already developed ripe conidia.

a faint trace of the place of former attachment can sometimes be observed on its wall. The mature conidium is oval in shape, thin walled, with a small apiculus $2\mu \times 3\mu$. (See p. 137, Fig. 2.) The contents are granular and of greyish colour. The average size of 148 conidia of the fungus growing on "wild" and cultivated hops in England was $27\mu \times 17\mu$[1].

The conidia, except those in early stages of development, become detached from the branches on contact with water; they are heavy and readily sink in water. The only method of germination observed by us is by the production of zoospores which escape from the conidium into the surrounding water. The time required by the mature conidium for zoospore-formation has been several times noted by us and has varied from one hour to three hours. Previous to germination the contents of the conidium become faintly segmented. Very suddenly the first zoospore escapes by squeezing through a small aperture at the papilla end of the conidium, and is followed by others in rapid succession. Often there is a moment's pause before the zoospore swims away. On rare occasions one, or even two, zoospores left in the conidium after release of the others, have been observed in active movement within the wall of the spore and were apparently unable to escape. Free-swimming zoospores may often be found attached in pairs by their posterior ends, showing that segmentation of the contents of the conidium is not always perfect, even at the time of their release. The number of zoospores produced by different conidia varies considerably, the following numbers having been observed in several instances: 4, 5, 6, 7[2]. The zoospores measure $12–14\mu \times 8–10\mu$; they are irregular in outline or oval, tapering to the posterior end, colourless, commonly with two vacuoles, and when in motion often revolve around their longer axis[3]. From examination of zoospores in movement, it appears that they are somewhat flattened. After a short free-swimming period the zoospore comes to rest, becomes circular in outline and then measures about 10μ in diameter.

In early autumn the fungus forms oospores within the tissues of the hop leaf. By moistening and scraping the brown, angular patches caused by the fungus (or the larger areas due to coalescence of such patches)

[1] Kingo Miyabe and Takahashi (19) give the measurements for the conidium as $22–26\mu \times 15–18\mu$.

[2] It is believed, however, from our observations that a larger number of zoospores than seven is not uncommonly produced, although the actual number was not counted. Miyabe and Takahashi (19) give the number as "about eight."

[3] The number of flagella was not determined. Miyabe and Takahashi (19) describe the zoospore of *Peronoplasmopara Humuli* as "kidney-shaped with two cilia attached to its lateral side."

on the lower surface of the leaf, numbers of oospores can be obtained for examination in a drop of water. The oospore is provided with a thick, smooth colourless wall. The contents are granular, of a grey-brown colour, and in some cases a lighter or darker area, circular in outline, is present. In most cases the shape is globular, but is sometimes oval, probably as a result of the oospores having been crowded in groups. (See p. 137, Fig. 2.) The average size of 104 oospores was $38\mu \times 37\mu$,

Fig. 1. Conidiophores showing types of branching:
1. On cultivated hops (*H. Lupulus*) from a farm in Kent. (× 250.)
2. On cultivated hops (*H. Lupulus*) from Wye College hop-garden. (× 250.)
3. On perennial nettle (*U. dioica*) Westwell, Kent. (× 250.)
4. The ultimate branches (*U. dioica*). (× 500.)
5 and 6. The ultimate branches (*H. Lupulus*). (× 500.)

the limits of length and breadth (for globose and oval spores) being $28–50\mu$ in length and $20–48\mu$ in breadth. The above refers to oospores found in living leaves of the hop in England. The average size of the oospore in three herbarium specimens of the fungus from Japan (on wild and cultivated hops) was found to be $33\mu \times 32\mu$, with a variation of $24–42\mu$ in length and $23–42\mu$ in breadth. In a herbarium specimen of

the fungus on the wild hop from the United States (Wisconsin) the average size of the oospore was $34\mu \times 33\mu$ with a variation of $26–44\mu$ in length and $26–43\mu$ in breadth.

Examples of the Downy Mildew of the Nettle, *Peronospora Urticae* (Lib.) de Bary, were collected from various localities in Kent, and from

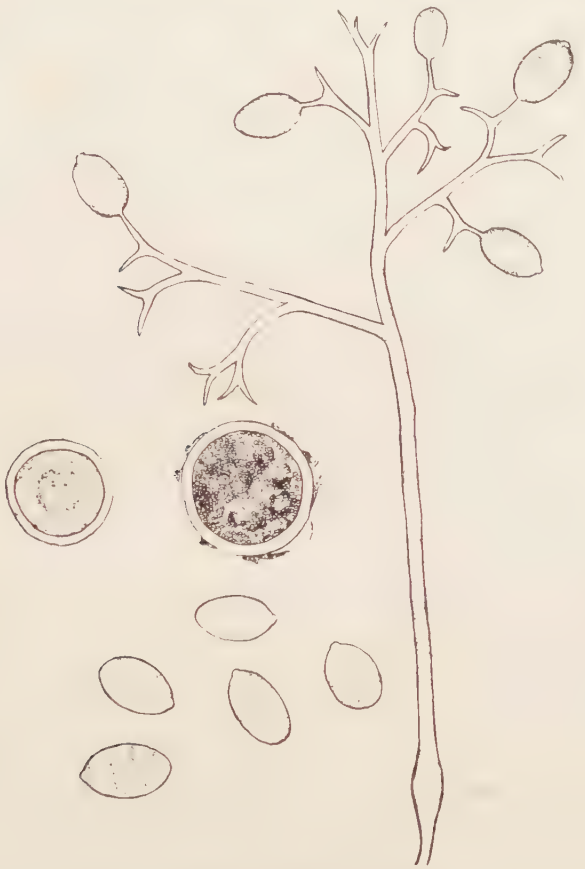


Fig. 2. Mature conidiophore of the Hop Downy Mildew (*Pseudoperonospora Humuli* (Miyabe and Tak.) Wils. with mature and immature conidia. Below, oospores and ripe conidia. Cultivated Hops, Wye College, Kent, 1924. ×400.

one locality in Middlesex, on *Urtica dioica* and *U. urens*, and examined microscopically. The general appearance of this mildew on the leaf of the Common Nettle (*U. dioica*) is shown in Plate VII, Fig. 2; small dark angular spots are formed on the upper surface of the nettle leaf, and on the under surface of these the conidiophores are produced. No

morphological difference could be found in the conidial stage, the size and manner of branching of the conidiophores and the peculiar shape of the conidium were the same as in the fungus on the hop. (See p. 136, Fig. 1.) The average size of 105 conidia was $27\mu \times 18\mu$, with the limits for length $22–40\mu$ and for breadth $14–22\mu$. The germination of the conidium was found to be by zoospores produced exactly in the manner described above for the mildew on the hop[1].

A difference was found in one character of the oospore, viz. the size. The average size of 80 oospores in the fungus on *U. dioica*[2] was $30\mu \times 29\mu$, the limits of length and breadth (for globose and oval spores) being $16–38\mu$ in each case. The tables given below (see p. 140) show all the *data* as to the size of the conidium and oospores in the fungus on the Hop and on the Nettle.

There is one fact which may be pointed out here. Reference to Tables I and II shows clearly that no difference exists in the size of conidium or oospore of the fungus on hops in the Experimental Hop-garden at Wye and the fungus on wild hops in other parts of England. If the fungus in the first named locality proves to be different in pathogenicity—a point discussed below on p. 148—it would constitute a biological, and not a morphological entity.

If we average our 225 measurements of the oospore of the fungus on the Hop, found in the English, Japanese and American examples, we find the size to be $35{\cdot}8\mu \times 34{\cdot}4\mu$, with the limits of $24–50\mu$ in length, and $20–48\mu$ in breadth[3]. According to our measurements, then, the mildew on the Nettle differs in having an oospore of a smaller size, 80 measurements averaging $30\mu \times 29\mu$, with the limits of $16–38\mu$ in length and breadth. Let us now see what other observers have recorded with respect to the characters of the mildew on the Nettle.

The fungus was first known to science in 1846, when Berkeley[4](1), calling the fungus "*Botrytis Urticae* of Mlle Libert," mentioned that it occurred in the autumn at Tansor in Northamptonshire.

[1] The following numbers of zoospores were observed: 5, 6, 9, 11, 14. This mode of germination had previously been observed in 1920 by Dr H. Wormald and the senior writer.

[2] No oospores were observed on *U. urens*, in the few examples examined.

[3] Kingo Miyabe and Takahashi give ((19), p. 3 (reprint)) the size of the oospore as "spherical, $28–34\mu$ in diam.," and state the "wall of the oogonium is persistent and loosely surrounding the oospore. The diameter of the oogonium is about 40μ." In the diagnosis given at p. 5 (reprint), the size of the oospore is given as "$25–40\mu$ in diam."

[4] In 1851 Berkeley and Broome (2) gave a diagnosis of the fungus, still called *Botrytis Urticae*, in which the following occurs: "flocci [*i.e.* conidiophores] loosely divided above, branches forming an acute angle, extreme ramuli simple or forked, sometimes curved, very rarely inflated." There seems no doubt that with regard to the character last men-

In the diagnosis given by de Bary in 1863 (10), who placed the fungus in *Peronospora*[1], the occurrence of oospores is mentioned, but no measurements either of these or of the conidia are given. Berlese and De Toni (3) and Fischer (12) give only the measurements of the conidia, viz. "22–26 $\mu \times$ 17–20 μ" and "aver. 20 $\mu \times$ 26 μ," respectively. Berlese[2], a monographer of the *Peronosporaceae* (4, 7) gives figures of conidia and oospores, with a diagnosis containing the following measurements: conidia, 20–27 $\mu \times$ 18–22 μ; oospores globose, 30–32 μ diam. Massee gave in 1891 (17) the following measurements: conidia 20–28 $\mu \times$ 17–21 μ; oospores globose, 21–25 μ diam., but in a later work in 1913 (18) changes the size of the oospores to "21–32 μ diam." Gäumann, who has studied closely (15) the Swiss species of *Peronospora*, gives for *P. Urticae* on *Urtica urens* 30 μ as the "mode" for the length of the conidium, with the limits 20·8–38·4 μ, and 22 μ as the "mode" for the width, with the limits 12·8–28·8 μ. Jaczewski gives (15*a*) the size of the conidia as 20–27 $\times$ 17–22 μ, of the oogonia as 35–45 μ in diam., and of the oospores as 25–32 μ, with 25 μ as the average[3].

Summarizing the matter, it must be conceded that from the morphological standpoint the fungus on the Nettle cannot in its conidial stage be separated from the fungus on the Hop. As regards oospore characters, the size of the oospore in the Nettle has been recorded by the observers noted above as from 21 to 32 μ, while in the specimens which we have examined, the size varied from 16 to 38 μ, with an average of 30 $\mu \times$ 29 μ. The size of the oospore of the fungus on the Hop was (as noted above) found to vary from 20 μ to 50 μ, averaging 35·8 $\mu \times$ 34·4 μ. The degree of systematic importance that is to be attached to such a difference in size (if found constant) must be left to the judgment of the monographer of the genus.

tioned Berkeley and Broome were in error; the ultimate branches of the conidiophore are not inflated, but are as shown in our figures given on p. 136. De Bary in 1863 (10), after giving a diagnosis, remarks: "Descriptio a cll. Berkeley et Broome data ad nostram non quadrat"; this would seem to refer to the above discrepancy, as de Bary describes correctly the ultimate branches as "subulati arcuati saepe deflexi."

[1] In mycological literature generally (*e.g.* Saccardo, *Sylloge Fungorum*), de Bary is quoted as being the first author to place, in 1863, the present species in the genus *Peronospora*. In reality, as Berkeley (*Outlines of British Fungology*, 349 (1860)) and Cooke (*Microscopic Fungi*, p. 216 (1865); *Handbook of British Fungi*, II, 595 (1871)) have pointed out, Caspary at an earlier date viz. in 1855 (in *Ber. Verhandl. Königl. Akad. d. Wissensch. Berlin*, 1855, p. 330) transferred the *Botrytis Urticae* Libert MSS. to *Peronospora*.

[2] Berlese, in his *Icones Fungorum* (4) represents the conidium of *P. Urticae* as without an apiculus (the base of the conidium, however, is depicted as somewhat apiculate). In the diagnosis given later (7) by this author, there is also no mention of the apiculus.

[3] We are indebted to Miss E. M. Wakefield for kindly sending us the above extract from the Russian.

Table I.

Measurements of conidia.

Host plant	Source	Limits in μ Of length	Of breadth	No. measured	Average in μ
Cultivated Hop (*Humulus Lupulus*)	College hop-garden, Wye, Kent	24–32	15–20	24	27 × 18
	Other hop-gardens in Kent	22–34	14–22	42	28 × 18
	All above sources, Oct.-Nov. 1924	22–34	14–22	66	27 × 18
"Wild" Hops (*H. Lupulus*)	Waste ground, Twickenham, Middlesex	22–27	14–18	18	24 × 16
	Hedge, Bickington, N. Devon	22–32	16–20	25	28 × 18
	Hedge, Westwell, Kent	22–32	15–20	39	26 × 17
	All above sources, Oct.-Nov. 1924	22–32	14–20	82	26 × 17
Perennial Nettle (*Urtica dioica*)	Various sources; Kent and Middlesex, Oct.-Nov. 1924	22–40	14–22	94	27 × 18
Annual Nettle (*U. urens*)	Marden, Kent, Oct.-Nov. 1924	24–30	16–19	11	28 × 17
Wild Hop (*H. americanus*)	Collected Lynxville, Wis. U.S.A. Sept. 9, 1915*	22–34	14–20	65	28 × 17
Wild Hop (*H. Lupulus* var. *cordifolius*)	Morioka, Japan, July 1906*	22–34	16–21	46	27 × 18
Cultivated Hop	Sapporo, Japan, June 1913*	22–30	14–20	21	26 × 17

* Dried herbarium specimens.

Table II.

Measurements of oospores.

Host plant	Source	Oospores globular and oval Limits in μ Of length	Of breadth	No. measured	Average in μ
Cultivated Hop (*Humulus Lupulus*)	College hop-garden	30–50	28–46	54	39 × 38
	Other hop-gardens in Kent	28–46	28–44	19	39 × 37
	All above sources	28–50	28–46	73	39 × 38
"Wild" Hop (*H. Lupulus*)	Waste ground, Twickenham, Middlesex	—	—	—	—
	Hedge, Bickington, N. Devon	42	40	1	—
	Hedge, Westwell, Kent	30–48	20–48	23	37 × 35
	Hedge, Hastingleigh, Kent	30–44	24–40	7	35 × 32
	All above sources	30–48	20–48	31	37 × 34
Perennial Nettle (*Urtica dioica*)	Various sources; Kent	16–38	16–38	80	30 × 29
Annual Nettle (*U. urens*)	—	—	—	—	—
Wild Hop (*H. americanus*)	Herb. specimen, Lynxville, Wis. U.S.A. Sept. 9, 1915	26–44	26–43	58	34 × 33
Wild Hop (*H. Lupulus* var. *cordifolius*)	Herb. specimen, Morioka, Japan, July 1906	32–40	30–40	2	—
Cultivated Hop	Herb. specimen, Sapporo, Japan, June 1913	25–40	35–38	35	33 × 32
Cultivated Hop	Herb. specimen, Sapporo, Japan, July 1905	24–42	23–42	26	33 × 32

The question of the systematic position of the Nettle Downy Mildew may be considered here. The discovery noted above, that the conidium, on germination, forms zoospores, necessitates the removal of the species from *Peronospora*, a genus defined by modern systematists, *e.g.* Berlese and De Toni (3), Schroeter (23), Berlese (5), Massee (18), Jaczewski (15*a*), Wilson (24) as composed of species the conidia of which germinate by a germ-tube. For reasons which appear to be valid, Wilson (24) rejects the generic name *Peronoplasmopara* (proposed as a sub-genus by Berlese (6) in 1901 and raised to generic rank by Clinton (8) in 1905) in favour of *Pseudoperonospora*, a genus founded by Rostowzew (20) in 1903, to which he transferred (26) the species *Pseudoplasmopara Humuli* Miyabe and Takahashi—a fungus clearly co-generic with *Peronospora Urticae*. The writers consider therefore that the correct name for the Nettle Downy Mildew is *Pseudoperonospora Urticae* (Libert)[1].

In view of the close relationship of the two fungi on the Nettle and Hop, and having regard to the economic consequences involved, it became highly desirable that cross-inoculation experiments should be carried out as soon as possible.

5. Inoculation experiments.

(*a*) *From Hop to Annual Nettle* (Urtica urens).

Exp. 1. Naturally infected leaves of the cultivated Hop were obtained from the College Experimental Hop-garden on Oct. 11, and in order to secure a plentiful growth of conidiophores the leaves were kept in a damp atmosphere for two days. A suspension of conidia in sterile distilled water in a flamed watch-glass was made on Oct. 13. After $1\frac{3}{4}$ hours the inoculations were made, using drops of the water which then contained numerous free-swimming zoospores. Fourteen of the youngest expanded leaves on ten healthy plants of *U. urens* (which had been grown in pots in a cool greenhouse) were used for this purpose. The liquid was drawn up by a sterilised capillary tube and run on to the lower surface of the leaf on one side of the mid-rib only. The remaining liquid was examined and the zoospores were observed to be still active. Each plant was covered by a bell-jar for three days after inoculation, but in two instances the bell-jars remained over the plants until the close of the experiment. On the 6th day (Oct. 19) after inoculation, several dark grey tufts of conidiophores were found on the inoculated side of the mid-rib on two leaves of the plants which had been kept covered and small dark areas, corresponding to the position of the conidiophores, were noticeable on the upper surface of both leaves. On the same date two leaves on two plants which had been uncovered on the third day also bore tufts of conidiophores, these were lighter grey, smaller and less conspicuous. The remaining six plants (ten leaves) never became infected.

Exp. 4 *a*. A suspension of zoospores, obtained from naturally infected hop plants in a commercial nursery on a farm in Kent was used for the inoculation of 16 leaves

[1] Further investigation is necessary to determine whether *P. Humuli* should be sunk in this species, or perhaps given varietal rank under it.

on six plants of *U. urens* on Oct. 24. After ten days (Nov. 3) one inoculated leaf showed tufts of conidiophores, and two other inoculated leaves showed small dark areas similar to those produced by the present fungus but on which no conidiophores could be found. All the plants had been covered with bell-jars and remained so for some time longer but no further infections were observed.

Exp. 7 *a*. On Nov. 7 the hop-leaves mentioned below in Exp. 2 were brought into the laboratory and a suspension of conidia was obtained from them. Comparatively few zoospores resulted, probably owing to the fact that the conidia were old. Two pot-plants of *U. urens* were inoculated with this suspension, and the plants were kept covered with bell-jars in the laboratory (temp. 54–56° F.). Six leaves on one plant were inoculated; these gave negative results. Nine leaves of the other plant were inoculated, and only one leaf became infected.

(*b*) *From Hop to Perennial Nettle* (Urtica dioica).

Exp. 7 *b*. The same suspension of conidia as was obtained in Exp. 7 *a* (above) was used (on Nov. 7) to inoculate two plants of *U. dioica*[1], which were kept under the same conditions as the plants of *U. urens* in Exp. 7 *a*. Of the eight leaves inoculated on one plant, one became infected; of the seven leaves inoculated on the other plant, three showed tufts of conidiophores. The control half areas in each case, and all the other leaves on the plants, remained healthy.

Exp. 4 *b*. The same suspension of zoospores as was obtained in Exp. 4 *a* was used to inoculate twelve of the youngest expanded leaves on six plants of *U. dioica*. The plants were kept, covered with bell-jars, in an unheated greenhouse. On the 10th day (Nov. 3) after inoculation, one inoculated leaf developed the fungus. No further infections took place.

(*c*) *From Perennial Nettle* (U. dioica) *to Hop*.

Exp. 2. A suspension of conidia was made on Oct. 15 from the Downy Mildew collected on leaves of plants of *U. dioica* growing by a roadside hedge at Wye; the liquid containing active zoospores was placed on one side of the mid-rib on the lower surface of four leaves of the Hop. The hop plant in question was growing in the Experimental Hop-garden, at Wye College, and the leaves were those of the basal side-shoots (of a few inches in length) springing from the main stem ("bine") which had been cut back earlier in the season. The plant was kept permanently covered, after inoculation, with a "cloche." No infection had occurred within a week; on the 12th day (Oct. 27) three of the inoculated leaves bore tufts of conidiophores on the inoculated half only, while one inoculated leaf showed a tuft of conidiophores on the control side only. The weather during the period was changeable, being mild with occasional very cold days. One of the three leaves above mentioned was so heavily infected that the one half of the lower surface appeared greyish-black from the dense mass of conidiophores and the upper surface showed the characteristic brown areas.

[1] The plants of *U. dioica* used in this experiment and also in Exp. 4 *b* had previously been potted up and kept in a cold greenhouse. Previous to their use, it had been noticed that some of the older leaves of a few of the plants showed signs of natural infection by the Downy Mildew; such leaves were removed before the others were inoculated, and no subsequent natural infections were observed.

Exp. 5 *a*. A suspension of zoospores, obtained from the Downy Mildew growing on stock plants of *U. dioica* in a separate greenhouse, was used on Nov. 4 to inoculate twenty leaves on two pot-plants of the Hop brought into a cold greenhouse. No infection occurred. It is possible that in this case the leaves were too old for infection.

Exp. 5 *b*. The same suspension was used to inoculate, in the one case four leaves, in the other three leaves, of two young basal shoots arising from the cut-back stems (bines) of two hop plants in the Experimental hop-garden. The plants were kept covered with bell-jars. The shoot with four leaves was destroyed by slugs; on the second shoot, one of the inoculated leaves became infected by the 5th day. All the control areas remained free and the six other leaves on that shoot were healthy.

Exp. 6. Six seedling hops in the cotyledon stage were inoculated on Nov. 4 each on one cotyledon only, with a suspension of zoospores obtained from the same source as in Exp. 5 *a*. The inoculated plants were kept, covered over with inverted beakers, in a cold greenhouse. No infections resulted[1]. The inoculations were repeated on all six plants on Nov. 7, with a fresh suspension of zoospores obtained from *U. dioica*. No infections resulted. In this case the temperature of the greenhouse never sank to below 45° F.[2]

Exp. 8 *a*. Twelve leaves from young hop shoots in the open were collected on Nov. 7 and placed on damp filter paper in Petri dishes in the laboratory. These were all inoculated on the under surface with a suspension of zoospores obtained from the Downy Mildew on *U. dioica*. In every case the control half area of the under surface of the leaf was wetted with distilled water[3]. Two of the leaves turned brown and were removed from the experiment. Of the remaining ten leaves four became infected on the areas inoculated, and six remained uninfected. The mildew was noticed first on the 10th day (Nov. 17). Scattered around the normal tufts were small groups of three or four (or even single) conidiophores; these were assumed to be instances of weak infection. None of the control areas became infected.

Exp. 8 *b*. The same suspension (described above) was used to inoculate a shoot bearing five leaves (all inoculated) which was growing in the hop-garden. After inoculation, the shoot was covered with a bell-jar. No infection took place, possibly owing to the low temperatures which occurred shortly after the inoculations were made[4].

Exp. 8 *c*. The same suspension was also applied to three leaves of a shoot which was removed from a plant in the hop-garden, brought into the laboratory and kept in a pot of moist sand. The leaves were inoculated on Nov. 7, and the shoot was then kept covered over with a bell-jar. One of the three leaves became infected, the other

[1] The temperature during the night of Nov. 4 sank to as low as 35° F.

[2] The cotyledons of actively growing seedling hop plants are susceptible to the Hop Downy Mildew; it is possible that the failure of the inoculations in this case was due to the fact that the seedlings (sown out of their proper season) were not actively growing, and indeed never advanced beyond the cotyledon stage.

[3] This treatment was given in order to secure favourable conditions for the germination of any conidia that might by chance have been present on the control areas of the leaves.

[4] The minimum temperatures on the grass for the seven nights following the inoculation were as follows: Nov. 7, 43° F.; Nov. 8, 48°; Nov. 9, 31°; Nov. 10, 32°; Nov. 11, 36°; Nov. 12, 46°; Nov. 13, 45°.

two and all the control areas remaining healthy. In this case also, the control areas of the inoculated leaves had been previously wetted.

Exp. 8 *d*. The same suspension was used to inoculate five leaves of another shoot of a hop plant kept under similar conditions in the laboratory. In this case three leaves remained healthy and the other two became infected on both the inoculated and control areas to about the same extent, so that no inference could be drawn.

In reviewing the evidence summarized on p. 145, certain circumstances must be taken into account. In the first place the time of the year (October and November) was unfavourable for obtaining positive results, many of the experiments being carried out at so late a season that the temperature, particularly at night, was very low[1]. The failure of the control leaves in some experiments was consequent on the forced use of late shoots arising from hop plants which probably had previously been attacked by the mildew. Owing to the economic importance of the disease and its epidemic spread during 1924 there was the imperative necessity that all steps should be taken to investigate its origin without waiting for a more suitable season of the year and for a supply of young uncontaminated plants for inoculation.

Making allowance for the facts mentioned above, there does appear to be sufficient evidence indicating that the Downy Mildew of the Hop can pass to the Nettle, and, probably, *vice versâ*. Taking the experiments where the fungus was transferred from the Hop to *Urtica urens* (which are free from the objection that controls became contaminated) six out of the 45 leaves inoculated became infected, *i.e.*, 13·3 per cent. Similarly, in the cases where the transference was from the Hop to *U. dioica*[2], five out of the 27 inoculated leaves became infected, *i.e.*, 18·5 per cent.

In the cases where the fungus was transferred from the Nettle to the Hop, if we accept the evidence of all the experiments as trustworthy, we have 11 leaves infected out of 62 inoculated, or 17·7 per cent.

Certain of the experiments, *e.g.* 8 *a*, seem to be free from objection,

[1] The minimum night temperature on grass at Wye from Nov. 1 to Nov. 17 varied between 48 °F. and 24° F.

[2] In the case of the plants of *U. dioica* which were used, there was the possibility (see above, p. 142 (footnote)) that previous to use conidia might have been carried to the leaves and, waiting for moisture in which to germinate, might have been the agent of infection when the water with zoospores was applied. (In this connection it may be mentioned that Doran (*Bull. Torrey Bot. Club*, XLIX, 11, 313–336 (1922)) states that precipitated moisture is essential to the germination of conidia of *Peronospora pygmaea*. If this hypothesis were correct, however, the negative results obtained on many of the inoculated leaves would be hard to explain. In certain of the experiments (Nos. 8 *a*, 8 *c*) the control half of the leaf was wetted as an additional safeguard.

and appear to show that the fungus can pass from the Nettle, *U. dioica*, to the Hop. It will be necessary, however, to wait until further investigations are made, using material free from all suspicion of previous contamination, before the positive statement can be made that *Humulus Lupulus* is one of the host-species of *P. Urticae*.

The degree of infective capacity indicated by the percentages of infections given above, when the fungus is transferred from one host-genus to another is low, but may possibly be accounted for (leaving out of consideration the probably unfavourable conditions of temperature under which the experiments were carried out) by the fact that, as Gäumann (14), Wartenweiler (23*a* & *b*) and Schweizer (23*c*) have shown, specialisation of parasitism occurs in the *Peronosporaceae*.

No. of Exp.	Suspension of zoospores: From	Suspension of zoospores: To	Total No. of leaves inoculated	No. of leaves infected	No. of leaves where infection failed	No. of control areas which became infected	No. of leaves destroyed by insects, etc.
1	Hop	*Urtica urens*	14	4	10	0	0
4 *a**	„	„	16	1†	15†	0	0
7 *a*	„	„	15	1	14	0	0
			45	6	39	0	0
7 *b*	„	*U. dioica*	15	4	11	0	0
4 *b*	„	„	12	1	11	0	0
			27	5	22	0	0
2	*U. dioica*	Hop	4	3	1	1	0
5 *a*	„	„	20	0	20	0	0
5 *b*	„	„	3	1	2	0	4
6	„	„	12	0	12	0	0
8 *a*	„	„	10	4	6	0	2
8 *b*	„	„	5	0	5	0	0
8 *c*	„	„	3	1	2	0	0
8 *d*	„	„	5	2	3	2	0
			62	11	51	3	6

* In the experiments designated by the same integer the same suspension of zoospores was used.

† Two of the inoculated leaves showed small dark areas of leaf tissue which may possibly have indicated infection up to a certain stage of development.

6. The Nettle as a possible source of the disease.

If we consider that the evidence tends to show that the mildew on the Nettle is (notwithstanding its apparently smaller oospore) identical with the species on the Hop, a solution of a puzzling phenomenon may perhaps be forthcoming. There is no record in mycological literature of

the occurrence of any Downy Mildew on the Hop in Europe before its appearance in Kent (Wye) in 1900. No specimens exist in our National Herbaria at Kew and at South Kensington. The sudden appearance of the Hop Mildew in epidemic form on hops, wild and cultivated, in England in 1924 has therefore to be accounted for, and we can do so if we assume that under the exceptional weather conditions of the summer and autumn of 1924[1] the Nettle Mildew was able to pass on to the Hop. The Nettle mildew, according to Continental authorities, *e.g.* Fischer(12), Lind(16), occurs on Nettles from May or June to October, thus giving a long period for such an opportunity. With the theory in mind that the Nettle Mildew can pass on to the Hop, it is interesting to find that in other countries where the Hop has recently been found to be attacked, the Nettle Mildew is known to exist. During the past year "a *Peronospora*" has been observed on the Hop for the first time in Germany. This fact has been communicated to us in a letter by Dr Lang (of the Württembergische Landes-Anstalt für Pflanzenschutz, Hohenheim)[2]. The publication of the circumstances under which this outbreak has taken place will be awaited with interest; it may be noted here that the Nettle Mildew (*P. Urticae*) occurs in Germany (including the hop-growing districts) on both *U. dioica* and *U. urens*.

In the United States a Hop Downy Mildew was, as noted above (p. 122), reported as a new occurrence, from Wisconsin, by Dr Davis in 1909. The earliest record of *P. Urticae* in that country is by Harkness and Moore(13) in 1880, "on nettle, San Raf.," California[3]. Farlow, in his "Enumeration of the *Peronosporaceae* of the United States," published in 1883(11), includes *P. Urticae*, but states that he has not examined American specimens and gives de Bary's specific description[4], with "California (Harkness)" as the only locality known. G. W. Wilson, in 1908(25), in his "Studies in North American *Peronosporales*," gives a list of the host-plants of "the commonly recognised American species of the Order"; we find here *P. Urticae* de Bary recorded on *Urtica* sp.,

[1] On this theory it would be held that such a migration took place in England (though apparently on a very small scale) also in 1920, when a few hop plants in the Experimental Hop-garden at Wye became infected (see above, p. 123).

[2] Dr Lang writes: "Es ist richtig, dass wir in Württemberg heuer Schädigungen an Hopfen durch *Peronospora* sp. beobachtet haben. Der Bericht über unsere Erfahrungen ist aber noch nicht veröffentlicht, er wird jedoch im Laufe des Winters im Druck erscheinen."

[3] Mr S. A. Skan, of the Herbarium, Royal Botanic Gardens, Kew, who kindly transcribed for us the above record, adds the following note: "San Raf. is evidently an abbreviation of San Rafael, a place not far from San Francisco."

[4] We have not met with any specific description of *P. Urticae* by American mycologists.

U. gracilis Ait, and *Urticastrum divaricatum* (L) Kuntze (*Laportea canadensis* Gaud.). The most recent record known to us is in a letter[1] by Dr Davis, of Madison, Wisconsin, who reports that he has personally examined a specimen of *P. Urticae* on *Urtica gracilis* collected at Kirkland, Wisconsin. This record is particularly interesting from the fact that it comes from the State whence the Downy Mildew of the Hop was first recorded.

It appears therefore that the same theory could be held to account for the appearance of the Downy Mildew on the Hop in Germany and in the United States as has been advanced above when dealing with the recent outbreak in England, viz. that the Nettle Mildew has found a new host.

7. Conclusions.

The recent appearance in Japan and England of a new and serious disease caused by a Downy Mildew presents an interesting mycological problem. Professor Miyabe believes that in Japan the fungus has migrated from the wild hop of that country to the commercial hop-garden. In North America the fungus is believed to be native on the wild hop, and no serious damage has been reported as occurring on cultivated hops in that country. In England the occurrence of the disease in a mild form was observed on cultivated hops at Wye in 1920, and the intensity of the disease increased there in succeeding years. Had no further facts come to light, a theory supported by mere circumstantial evidence, might have been held that the disease had been imported on hop plants from Japan or America, which as a matter of fact had been obtained, for breeding purposes, from both these countries. In 1924, however, an epidemic outbreak occurred in England, totally unconnected with the original outbreak at Wye. The fungus was found, during the very wet autumn of 1924, on leaves and cones of "wild" hops in counties far away from cultivated hops, and also, in October and November, on leaves of cultivated hop plants in hop-gardens and hop-nurseries, quite generally dispersed in Kent and other hop growing counties.

Now, it is not possible, we consider, that the Downy Mildew can have existed, throughout the growing season, in the hop-gardens of this country without having attracted attention. Prof. Kingo Miyabe holds the same view with respect to the appearance of the disease in Japan.

[1] The contents of this letter was kindly communicated to us by Miss Vera K. Charles, of the U.S. Department of Agriculture.

1905 was the date when this disease began to attack cultivated hops in Japan, and 1924 when a similar occurrence took place in this country. How has this new disease originated? In Japan it is believed to have originated on the wild hop; in this country we can perhaps carry the explanation a step further. We have shown that the Downy Mildew of the Nettle, hitherto called *Peronospora Urticae*, is a member of the genus *Pseudoperonospora*, and that it is indistinguishable morphologically in the conidial stage from the fungus on the Hop; the only morphological difference that we have been able to detect is in the size of the oospore; there being a difference, in English material, of 8μ between the average measurements. The view that the Downy Mildew attacking the Nettle and the Hop may be the same species is greatly strengthened by the fact that inoculation experiments show that the fungus can pass from the Hop to the Nettle, and probably *vice versâ*. Accepting, then, the origin of the epidemic outbreak in 1924 of a downy mildew on hops in England as being due to a general transfer of the fungus from Nettle to this new host plant, what economic results may be anticipated? On the more optimistic view, we might hypothecate that it is only under exceptional circumstances such as the very abnormal wet weather conditions of the summer and autumn of 1924 that the Hop plant becomes liable to infection by the Nettle Downy Mildew, and further, that the fungus does not persist on the Hop plant. Against this view (if we accept the Nettle Mildew as the *fons et origo*) is the fact that the Downy Mildew appeared in the hop-garden at Wye in 1920, did not die out, but persisting through the abnormally dry season of 1921, established itself as a very serious disease, recurring annually. Forced, therefore, to find an explanation for this, we can advance alternative views. Possibly the majority of the hop varieties grown in the Experimental Garden at Wye may be specially susceptible to the Downy Mildew coming from the Nettle, thereby securing for the fungus a permanence which might possibly not obtain in the case of the ordinary commercial varieties. On the other hand, it may be that biologic forms of the Hop Downy Mildew exist with different degrees of pathogenicity. On this hypothesis we may be dealing with a biologic form (possibly introduced on plants imported from Japan or America) which has caused the serious disease noted for the past five years in the hop-garden at Wye, and which probably has been introduced to the East Malling Research Station, and thence to certain farms in Kent and Sussex; and with a second, more harmless biologic form—undoubtedly native to this country (whether specifically identical with the Nettle Downy Mildew or not)—which

may be more or less ephemeral and incapable of causing a serious disease of cultivated hops. If this were so, it is possible that in past years during very wet seasons, a widespread but short-lived occurrence of a "Downy Mildew" in hop-gardens and hop-nurseries may have taken place and escaped notice.

The more pessimistic view would be to suppose that in an evolutionary process that is taking place, the Downy Mildew of the Nettle in this country is producing new forms capable of infecting the Hop and destined to cause in commercial hop-gardens in the future a disease of serious economic importance. New diseases of cultivated plants appear to have arisen in this manner; for instance, the comparatively recent occurrence of the Wart-disease of the Potato is due, it is believed, to the fungus *Synchytrium endobioticum* transferring itself from some wild host-plant to the cultivated Potato.

The investigations which we intend to make next season in hop-gardens which were attacked by Downy Mildew during the epidemic of 1924 will, it is hoped, throw light on some of the above problems.

8. Summary.

1. The Downy Mildew of the Hop (*Pseudoperonospora Humuli* (Miyabe and Takahashi) Wils.) recorded in 1905 from Japan and in 1909 from the United States, and noted for the first time in Europe in the Experimental Hop-garden at Wye in 1920, occurred during the autumn of 1924 in Kent and other hop-growing counties, in epidemic form, in hop-gardens and hop-nurseries.

2. The above fungus has been found to be indigenous to this country.

3. A description of the fungus and the characteristics of the disease produced on cultivated hops are given; hypertrophied, spike-like basal shoots have been found under circumstances which suggest the existence of a perennial mycelium. No differences were found in the measurements of conidia and oospores in English, Japanese and American material.

4. The Downy Mildew of the Nettle, known as *Peronospora Urticae* (Libert) de Bary produces zoospores, and must therefore be referred to the genus *Pseudoperonospora*. It differs from the fungus on the Hop only in respect of the smaller size of the oospore.

5. Inoculation experiments have shown that the Downy Mildew on the Hop can be transferred to Nettles (*Urtica urens* and *U. dioica*); and probably, *vice versâ*.

6. Various hypotheses are advanced to account for the recent epidemic appearance of this fungus on cultivated hops in this country.

9. REFERENCES.

(1) Berkeley, M. J. (1846). *Journ. Hort. Soc. London*, I, 31.

(2) Berkeley, M. J. and Broome, C. E. (1851). Notices of British Fungi. *Ann. and Mag. Nat. Hist.* ser. 2, VII, 100.

(3) Berlese, A. N. et De Toni, J. B. (1888). *Phycomyceteae. Sacc. Syll. Fung.* VII, 257.

(4) —— —— (1898) *Icones Fungorum. Phycomycetes*, 39, tab. 63.

(5) —— —— (1900). Saggio di una Monografia delle Peronosporacee. *Riv. Pat. veg.* IX, 3.

(6) —— —— (1901). *Plasmopara cubensis* (B. et C.). *Riv. Pat. veg.* IX, 123.

(7) —— —— (1904). Saggio di una Monografia delle Peronosporacee. *Riv. Pat. veg.* X, 269.

(8) Clinton, G. P. (1905). In *Report Conn. Agric. Exp. Station* for 1904, 334.

(9) Davis, J. J. (1910). A New Hop Mildew. *Science*, n.s., XXXI, 752.

(10) De Bary, A. (1863). Recherches sur le développement de quelques champignons parasites. *Ann. sci. nat.* 4 sér. XX, 116.

(11) Farlow, W. G. (1883). Enumeration of the *Peronosporaceae* of the United States. *Bot. Gaz.* VIII, 328.

(12) Fischer, A. (1892). *Phycomycetes. Rabenh. Kryptogamen-Flora*, I. Abt. IV, 473.

(13) Harkness, H. W. and Moore, J. P. (1880). Catalogue of the Pacific Coast Fungi, 30. *California Acad. Sciences*, Feb. 2.

(14) Gäumann, E. (1916). Zur Kenntnis der *Peronospora parasitica* (Pers.) Fries. *Centralbl. f. Bakter. Parasit. u. Infektionskr.* II. Abt. XLV, 576.

(14*a*) —— (1918). Über die Formen der *Peronospora parasitica* (Pers.) Fries. Dresden.

(14*b*) —— (1918). Über die Spezialisation der *Peronospora Calotheca* de Bary. *Svensk Botan. Tidsk.*, Bd. 12, H. 4, 433.

(14*c*) —— (1918). Über die Spezialisation der *Peronospora* auf einigen Scrophulariaceen. *Annal. mycolog.* XVI, 189.

(15) —— (1923). Beiträge zu einer Monographie der Gattung *Peronospora. Beitr. zur Kryptogamenfl. Schweiz*, V, Heft 4, 302, Figs. 38, 152, 153.

(15*a*) Jaczewski, A. (1901). Materials towards a knowledge of the Fauna and Flora of the Russian Empire. Botanical Section. Part IV. (Moscow.) [Russian.]

(16) Lind, J. (1913). In *Danish Fungi in Herb. Rostrup*, 61.

(17) Massee, G. (1891). *Phycomycetes and Ustilagineae*, 124.

(18) —— (1913). *Mildews, Rusts and Smuts*, 7, 34.

(19) Miyabe, K. and Takahashi, Y. (1905–6). A New Disease of the Hop-vine caused by *Peronoplasmopara Humuli* n. sp. *Trans. Sapporo Nat. Hist. Soc.* I, Part II.

(20) Rostowzew, S. J. (1903). Beiträge zur Kenntnis der Peronosporeen. *Flora*, XCII, 405.

(21) Salmon, E. S. and Wormald, H. (1923). Three New Diseases of the Hop. *Journ. Min. Agric.* XXX, 430.

(22) Salmon, E. S. and Ware, W. M. (1924). An Endemic *Peronospora* on the Hop in England. *Gardeners' Chronicle*, LXXVI, Oct. 18.

[Throughout this paper the fungus on the Hop was by an error called *Peronospora* instead of *Pseudoperonospora.*]

(23) Schroeter, J. (1897). *Engler u. Prantl, Die natürlich. Pflanzenfam.* I. Abt. I, 113.

Fig. 2.

Fig. 1.

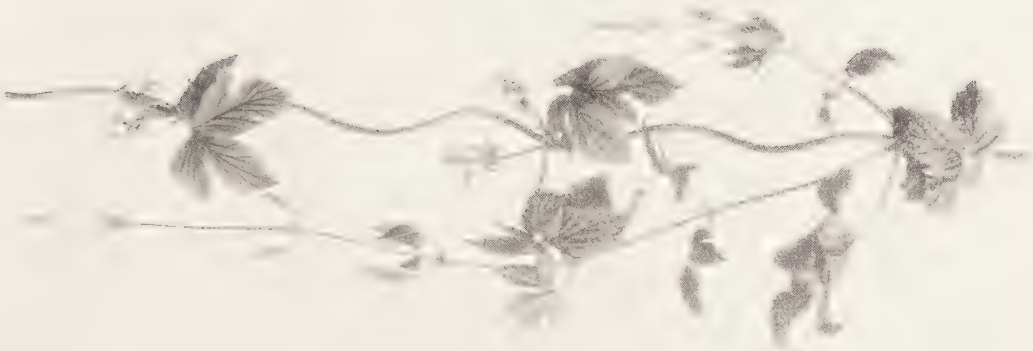


Fig. 4.

Fig. 3.

Fig. 6.

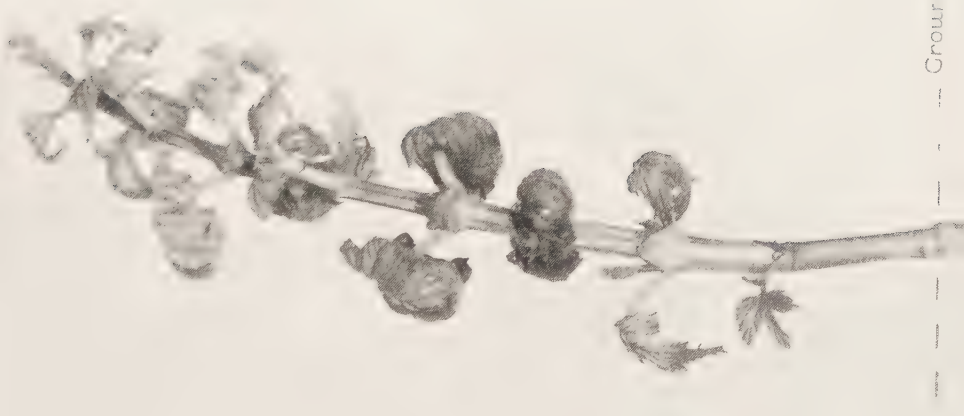


Fig. 5.

(23 *a*) Wartenweiler, A. (1918). Zur Biologie der Gattung *Plasmopara*. *Verh. d. schweiz. naturf. Ges.* 99, Zürich (1917), 223–224.

(23 *b*) —— (1918). Beiträge zur Kenntnis der Gattung *Plasmopara*. *Annales Mycologici*, xv, 495–497.

(23 *c*) Schweizer, J. (1919). Die kleinen Arten bei *Bremia Lactucae* Regel und ihre Abhängigkeit von Milieu-Einflüssen. *Verh. d. thurgausisch. naturforsch. Gesellsch.*, Heft 23.

(24) Wilson, G. W. (1907). Studies in North American *Peronosporales*, II. *Bull. Torrey Bot. Club*, xxxiv, 389, 412.

(25) —— (1908). *Ibid. l.c.* xxxv, 543.

(26) —— (1914). *Ibid. Mycologia*, vi, 194.

EXPLANATION OF PLATES VII, VIII AND IX

Fig. 1. Hop leaf showing the angular spots caused by the Downy Mildew. Nat. size. *Photo by* Dr H. Wormald.

Fig. 2. Leaf of *Urtica urens* showing dark angular spots caused by the Downy Mildew. This leaf was inoculated only on that side of the mid-rib, with zoospores obtained from the mildew on cultivated hops. ($\times 3$.) *Photo by* W. M. Ware.

Fig. 3. Cultivated hop. Stem, showing arrested growth at 5 feet from the ground. The tip is deformed and spike-like. Nat. size. *Photo by* Dr H. Wormald.

Fig. 4. Part of climbing stem (cultivated hop) showing a pair of normally developed lateral shoots (above) and two pairs of hypertrophied, spike-like shoots (25 June, 1923). Reduced. *Photo by* Dr H. Wormald.

Fig. 5. A hypertrophied, spike-like basal shoot (May, 1923) caused by the Hop Downy Mildew (the plant was a hybrid seedling (Ref. No. OR 51) of German parentage planted 9 years ago in the Wye College Hop-garden). $\times \frac{1}{16}$. *Photo by* Dr H. Wormald.

Fig. 6. Healthy basal shoot of same plant (May, 1923). $\times \frac{1}{16}$. *Photo by* Dr H. Wormald.

(*Received January 12th*, 1925)

EXPERIMENTS ON THE CONTROL OF WART DISEASE OF POTATOES BY SOIL TREATMENT WITH PARTICULAR REFERENCE TO THE USE OF SULPHUR

By

W. A. ROACH

(*Department of Insecticides and Fungicides*),

MARY D. GLYNNE, WM. B. BRIERLEY

(*Department of Mycology*)

AND

E. M. CROWTHER

(*Department of Soil Physics*).

Rothamsted Experimental Station, Harpenden.

(With 13 Text-figures and 2 folding Plates, X, XI.)

CONTENTS.

Introduction.

The problem of the control of Wart Disease of potatoes has hitherto been attacked along two fundamentally different lines. On the one hand there have been the selection and breeding of varieties of potato immune to *Synchytrium endobioticum*, methods which seek to evade rather than to destroy the disease-causing organism. On the other hand attempts have been made to discover some process of soil sterilisation whereby the resting sporangia of the fungus in the soil can be killed *in situ* so that susceptible varieties may again be cultivated on previously contaminated soil. The adoption of immune varieties offers an immediate control of the disease but is not necessarily a complete solution of the Wart Disease problem. The choice among immune varieties is limited, especially as practically all the established "First Earlies" are susceptible; and there is a widely held idea that many immune varieties are of inferior quality or produce smaller crops than the best susceptible varieties. In consequence growers in clean areas show, for most purposes, a marked preference for susceptible varieties and these are cultivated very widely both in this and other countries.

Again, many potato districts at home and abroad are still free from the disease save for small and isolated centres of infection and legislation restricting the movement and sale of potatoes from large areas of the clean land surrounding these nuclei has resulted in heavy financial losses to the growers. Such a position is well exemplified in the case of Lincolnshire where the presence of a few warted tubers in a town allotment made it impossible in 1924 to place on the French market many thousand tons of potatoes from the surrounding district. These small outbreaks occur frequently in allotments or cottage gardens and if it were possible to free the latter from disease the commercial growers in the surrounding regions would no longer be hampered by legal restrictions which interfere very seriously with the trade. Even therefore if a process of soil sterilisation were for financial or mechanical reasons impracticable for use on a large scale it would still be of the utmost value in the elimination of isolated areas of contamination.

The pot cultures and field experiments of 1920–23 were planned and carried out in equal part by Roach, Glynne and Brierley. In the spring of 1924 Miss Glynne was compelled by illness to relinquish her share in the field-work and the experiments of that year were carried out by Roach and Brierley. The work on soil acidity was done by Crowther and Roach.

It is such considerations as the above that have been the stimulus to investigations extending over two decades on fungicidal treatment of contaminated soil.

In this country the most extensive experiments were carried out by Malthouse(8) at the Harper Adams Agricultural College and the following substances were tested on a field scale: various forms of lime and sulphur, salt, soot, sodium borate, copper sulphate, potassium sulphide, ferrous sulphate, potassium bisulphite, calcium bisulphite, lead acetate, calcium hypochlorite, copper chloride, copper nitrate, copper arsenate, copper arsenite, potassium permanganate, potassium ferrocyanide, potassium ferricyanide, copper cyanide, ammonium sulphocyanide, and various patent mixtures such as Strawsonite and Nicholl's Remedy. The substances were incorporated with contaminated soil, singly and in different combinations, by several methods at varying times of the year, but all proved ineffective as soil fungicides against wart disease, or the remedy proved worse than the disease in that the crop was very adversely affected by the treatment. In his final report published in 1914 Malthouse states "Fungicides have given varying results and therefore may be considered to be quite valueless."

In 1914 Professor Eriksson(2) of Stockholm described experiments in which he claimed to have destroyed the resting sporangia of *Synchytrium endobioticum* in contaminated soil in small plots by means of a dilute solution of formalin (1 in 100 comm.) applied at the rate of 10 litres per square metre. At his request the English Board of Agriculture carried out experiments to test the effect of this treatment and also of the application of heavier doses of formaldehyde but reported "the results of these experiments are wholly unfavourable to the use of formaldehyde as a preventive of wart disease." The actual data given show that the treatment had had practically no effect on the incidence of the disease. Certain other substances were tested at the same time with similar results.

More recently Schaffnit and Voss(10, 11) in Germany have carried out extensive soil sterilisation experiments using the following substances: calcium cyanamide, kainit, sulphur, sodium cyanide, uspulum (an aqueous solution of mercury-chlorphenol), lysol, chromium acid carbonate, chromic oxide, flurasil (a silica fluor-zinc compound) and formaldehyde. These were incorporated with the soil at various seasons by several methods either singly or in different combinations and quantities, but all proved useless as they either failed to reduce the disease or greatly reduced the crop of potatoes.

In 1918 Gimingham and Spinks (3) carried out soil sterilisation experiments in 6 inch pots and found that the following substances in the proportions tested did not materially reduce the amount of disease: creosote, calcium hypochlorite, chloro-picrin, formaldehyde, chalk, cymene, sulphur dioxide and copper sulphate. A positive result was, however, obtained when contaminated soil was steamed for one and a half hours at 100° C., the disease in this case being apparently eliminated. In the Ormskirk potato trials of 1918 Snell (12) experimented with the steaming of infected soil in pots and reported "that the amount of wart on these plants was even greater than on the plants in (*b*)" (untreated soil). Snell's treatment however was by no means so thorough as that of Gimingham and Spinks.

In the same year Wart Disease was discovered in the Pennsylvanian regions of the United States of America and received a considerable amount of attention. Experiments on the sterilisation of soil in garden plots were carried out by Lyman, Kunkel and Orton (6) and in 1920 they reported as follows: "Exposure to live steam under the inverted pan for 85 minutes with a pressure of 90 lbs. at the gauge destroyed the potato wart fungus, in so far as could be determined by planting potatoes on the treated plots. The indications are that a combination of the formaldehyde solution and steam will be more effective than either treatment alone, since these preliminary tests show that the application of $1\frac{1}{2}$ pints of commercial (40 per cent.) formaldehyde in 70 gallons of water to 1000 square feet of surface of soil (about 0·54 pint per square foot) followed by the application of steam at 95 pounds gauge pressure for 30 minutes is efficacious." No further details of these experiments have been received but writing in October 1924 Hartmann and McCubbin (5) of the Pennysylvania Department of Agriculture state "Many experiments have been carried out to test methods of destroying the fungus in the soil and a few of these have shown some merit."

Thus towards the end of 1920 the outlook was far from hopeful; many of the obvious fungicides had been tested and all the results had been inconclusive or negative. It was at this point that the experiments about to be described were undertaken. When the experimental methods that had been used in the previous trials were considered critically it seemed very probable that much of the failure to achieve positive results could be attributed to faulty technique, and, more particularly, to lack of thorough incorporation of the fungicides with the soil.

The sporangia of the fungus exist generally scattered through contaminated soil with here and there a nidus of more intensive contamina-

tion where warts have rotted *in situ*. Since any single sporangium can, under favourable conditions, give rise to the disease it is imperative that all the sporangia in the soil be destroyed in order to render this free of contamination. To be effective any toxic substance or process must therefore permeate the soil completely. It would seem that this result can only be attained by a combination of thorough incorporation of the substance with the soil and of its diffusion either in solution or in gaseous state to the interior of lumps of soil. Since soil adsorbs many substances from solution and from gaseous mixtures the degree of penetration of a lump of soil by any given substance is likely to be very limited. Hence it is highly desirable that the soil be in a finely divided state in order to reduce the "radius" of each lump as much as possible, so that the toxic substance shall have the smallest possible distance to travel by diffusion.

It is unlikely that application of fungicides by dusting on the sets or in the drills at planting, or on the soil surface, or even applied by hand digging or any form of ploughing or harrowing would produce the necessary intimate mixture. Such methods would probably leave numbers of sporangia in contaminated soil quite untouched by the fungicide, however heavily this might be applied. The hypothesis that a considerable part of previous work had been unsuccessful because the substances used had not been brought into effective contact with every sporangium seemed the most feasible explanation of the variable results in view of the extremely toxic properties of many of the fungicides tested and of the quantities in which they had been applied. This hypothesis, in effect, meant that the toxic relation of many of the fungicides to the sporangia of *Synchytrium* had not really been examined, for the substances in many cases had probably never come into effective contact with the sporangia. To a certain extent therefore the problem was open from the beginning, for could the requisite intimacy of mixture be obtained many fungicides which previously had given negative results might, even in smaller quantities, prove toxic to sporangia when actually brought into contact with them.

The essential basis of the experiments described in this paper was therefore extremely thorough incorporation with the soil of the substance to be tested so that all the sporangia present might be brought into contact with the fungicide.

PRESENT WORK.

In ordinary fungicidal studies valuable information can be obtained and much time and expense saved by making preliminary tests *in vitro* of the toxic relations of possible fungicides to the disease-causing organism. Any substance or process which is non-toxic *in vitro* is unlikely to prove effective when applied on a field-scale and may therefore be eliminated from subsequent trials. In the case of Wart Disease this procedure has not been possible because of the absence of a suitable technique[1] due to the difficulty of inducing germination *in vitro* of more than a small fraction of any sample of resting sporangia. Thus even preliminary trials must be carried out by means of pot cultures which of necessity makes progress very slow since for each test potato plants have to be grown to maturity.

Since Wart Disease is scheduled by the Ministry of Agriculture and Fisheries under the Destructive Insects and Pests Act of 1907 (Wart Disease Order, 1923), severe restrictions are placed upon the movement of diseased plants or any other infective material, and in consequence our pot experiments for 1920–22 and later field trials had to be carried out in infected areas. They were therefore done at Ormskirk in the midst of one of the most intensively contaminated regions in the world and, to a lesser extent, during 1922–4 in a small contaminated garden-plot at Hatfield. The necessity of carrying out such investigations at a distance from Rothamsted greatly increased the difficulties of the work and without the generous help of Mr Bryan and his assistant Miss Whitehead at the Potato Testing Station, Ormskirk, many of our experiments would have been impossible.

Pot Experiments.

A. 1920.

In 1920 a preliminary set of experiments was carried out in ordinary brown earthenware pots of seven inches diameter[2]. Soil from the trial grounds at Ormskirk was treated in various ways and a tuber of a susceptible variety (Arran Chief) planted in each pot. The experiments were a failure owing to the almost complete absence of Wart Disease even in the control plants growing in untreated soil. At the time this was attributed partly to the unavoidable lateness of planting and partly to

[1] One of us (Glynne) has carried out a considerable amount of work on this problem and will discuss it in a later number of the *Annals of Applied Biology*.

[2] We are indebted to Mrs Snell for help in setting up and for a time attending to this series of pots.

the relatively small amount of growth of the plant possible in such small pots with the consequently reduced opportunities for infection. It was decided therefore to use much larger pots in succeeding experiments in which, it was thought, conditions of moisture, aeration, etc. would be less abnormal[1].

B. 1921.

In view of the failure of the previous year's experiments carried out in small pots the experiments of 1921 were done in large pots, 12″ × 12″ in size, and each holding sixty pounds of soil. These were specially made of glazed earthenware in order to prevent the absorption of the chemical by the pot itself and each had at the base a tubulure for drainage.

The experiments were designed to test the fungicidal effect of certain chemicals both alone and in conjunction with steam. The list of chemicals was drawn up in consultation with Mr Tattersfield (Head of the Department of Insecticides and Fungicides at Rothamsted).

Incorporation of Chemicals. Sixty pounds of heavily infected soil from the old trial ground at the Ormskirk Workhouse was weighed into each pot from a heap of soil which had been thoroughly mixed. The soil of each pot was then turned out on to a sheet of strong brown paper on a table and the chemicals were incorporated by hand. The small heap was then put six times through a sieve of one quarter inch mesh, care being taken that no two consecutive shovelfuls came from the same part of the heap. All signs of "stratification" due to unevenness of distribution had disappeared after the second sifting. After this thorough incorporation of the chemical the soil was returned to the pot. The sheet of paper was changed for each chemical.

Steaming of Pots. Two wooden boxes were constructed each to hold four pots. The latter were raised on pieces of wood 1″ × 1″ × 12″ to allow steam to circulate freely round the bottoms of the pots. A piece of iron gas tubing one inch in diameter was introduced through the side of each box so that its end was in the centre of the box. These two pieces of piping were connected by a T-piece, the remaining arm of which was connected to the boiler of a steam engine by means of flexible metal tubing. In the wooden lid of each box holes were made to allow of thermometers being pushed into the centres of the pots. Steam at a gauge pressure of 100 lbs. per square inch raised the temperature in the centres of the pots to 90–100° C. in about three hours. Owing to the

[1] See however "Infection Experiments with Wart Disease," by M. D. Glynne, *Annals of Applied Biology*, 1925, XII, 1.

unsuitability of the only thermometers available there was a possible error of about 5° C. in the readings due to the steam occasionally coming into contact with the mercury bulb. The pots were left for three and a half hours and the escape of steam prevented by covering the boxes with sacking. Although fairly dry soil was used it became sodden in the pots during steaming owing to condensation.

About one month after treatment of the soil a tuber of a susceptible variety was planted in each pot and the pots were kept in a glasshouse at Ormskirk and watered when necessary. Two series of experiments were carried out during the year, the method being the same in both cases.

Table I.

Pot Experiments on Soil Sterilisation.

First Series. Set up March 8–9, 1921. One tuber of a susceptible variety planted in each pot on April 5–6; half being set with the variety Eclipse and half with the variety Epicure. Plants lifted July 20, 1921.

Second Series. Set up July 25–29, 1921. One tuber of the very susceptible variety Arran Chief set in each pot, August 9–10. Plants lifted December 1–2, 1921.

Dose	Treatment Chemical	First Series		Second Series	
		Pots infected	Total pots	Pots infected	Total pots
—	Control. Untreated	3	7	5	10
—	Control. Steam only	0	8	0	5
·2	Formaldehyde	0	4	—	—
·2	,, +steam	**0**	**4**	—	—
·1	,,	—	—	0	3
·1	,, +steam	—	—	**0**	**3**
·05	,,	—	—	0	3
·05	,, +steam	—	—	**0**	**4**
·1	Bleaching powder	0	4	2	4
·1	,, +steam	**0**	**4**	0	3
·1	Sulphur	0	4	0	4
·1	,, +steam	**0**	**4**	**0**	**4**
·01	,,	—	—	3	3
·01	,, +steam	—	—	0	4
·1 (S basis)	Calcium polysulphide	0	4	0	2
·1 ,,	,, +steam	**0**	**4**	**0**	**2**
·1 ,,	Potassium polysulphide	—	—	1	3
·1	Chlordinitrobenzene	0	4	—	—
·1	,, +steam	**0**	**4**	—	—
·01	,,	2	4	—	—
·01	,, +steam	0	4	—	—
·1	Nitrobenzene	0	4	—	—
·1	,, +steam	**0**	**4**	—	—
·2	Acetaldehyde	2	4	2	3
·2	,, +steam	0	4	0	3
·075	Dichlorcresol	—	—	0	3
·0375	,,	—	—	1	4
·01	,,	—	—	3	3
·01	,, +steam	—	—	0	4

The results obtained in the two series of experiments are set out fully in Table I. Results of certain combined treatments which have little significance owing to the fact that either treatment alone prevents the appearance of the disease are printed in clarendon type. In most cases the results are not conclusive owing to the small number of pots used per test and to the small amount of infection which was found even in the control pots. Furthermore the results in the two series are not quite concordant; cases in which the disease was present however may be regarded as definitely proving that the particular treatment had not killed the sporangia of the pathogen, *Synchytrium endobioticum*, present in the soil. Thus treatment with ·1 per cent. bleaching powder, ·01 per cent. sulphur, ·1 per cent. potassium polysulphide, ·01 per cent. chlordinitrobenzene, ·2 per cent. acetaldehyde and ·0375 per cent. and ·01 per cent. dichlorcresol appeared to be useless in the control of Wart Disease. There are indications however that the amount of disease was reduced by formaldehyde, ·1 per cent. sulphur, ·1 per cent. calcium polysulphide, ·1 per cent. chlordinitrobenzene, ·1 per cent. nitrobenzene and ·075 per cent. dichlorcresol. Certain of these treatments were selected for more detailed study later.

Table II.

Pot Experiments on Soil Sterilisation arranged to show Effect of Steam.

Details as Table I.

Dose %	Treatment Chemical	Steamed		Unsteamed	
		Pots infected	Total pots	Pots infected	Total pots
	Untreated	—	—	8	17
	Steam alone	0	13	—	—
·01	Chlordinitrobenzene	0	4	2	4
·2	Acetaldehyde	0	7	4	7
·01	Sulphur	0	4	3	3
·1	Bleaching powder	0	3	2	4
·01	Dichlorcresol	0	4	3	3
	Totals	0	22	14	21

In Table II the results are arranged to show the effect of steaming the soil. It seems clear that this treatment as applied in this particular case was completely successful. Thus with steam alone there was a complete absence of infection in 13 pots whilst with untreated soil disease was present in 8 pots out of 17. Again when pots were treated with various chemicals infection was present in 14 pots out of 21 whereas the same treatments plus steam gave an absence of disease in all the

22 pots. Summarising these results, disease was present in 22 out of 38 unsteamed pots whereas there was a complete absence of disease in 35 corresponding steamed pots.

C. 1922.

In 1922 pot experiments were again set up as described on p 158, the aims being to confirm the results of 1921 and to test certain new substances. The pots were placed in the open at Ormskirk but owing to the impossibility of giving them continuous personal attention the soil became waterlogged and the experiments failed. By this time the difficulties of ensuring reliable results in pot experiments even under favourable conditions had become so obvious that the method of pot culture was discontinued and all subsequent work was carried out directly in the field. The principal difficulty in the method of pot culture hinged upon the fact that even strongly growing plants of susceptible varieties growing in intensely contaminated soil often remained free from Wart Disease so that apart from the use of impracticably large numbers of plants one could not rely upon one's results[1].

Field Experiments.

A. Ormskirk, 1922.

The experiments were planned to test the effect in the field of certain of the chemicals which appeared to give the most promising results in the pot cultures. The actual plots were set out as shown in Fig. I. By making each treatment overlap another one a convenient arrangement was obtained whereby the chemicals were tested in pairs, each member of the pair being tested separately with and without lime, and in combination with its fellows with and without the addition of lime. The field therefore was divided into six large blocks separated by paths 2 feet wide. Each block was subdivided into nine plots (8′ × 8′). Each member of a pair of substances was applied to four of these plots having one point in common, as indicated by the shading in Fig. 1. This arrangement, though not perfect, was convenient for the present purpose. (The scheme was not used for block 31–51.) Each small plot contained forty potato plants. The quantities and combinations of substances tested are shown in the accompanying table.

Method of Incorporation of Chemicals with Soil. In the pot experiments the method of hand mixing with subsequent repeated sifting was

[1] One of us (Glynne) investigated the pot culture method in its relation to Wart Disease and has published certain of her results in the previous number of this *Journal* (4).

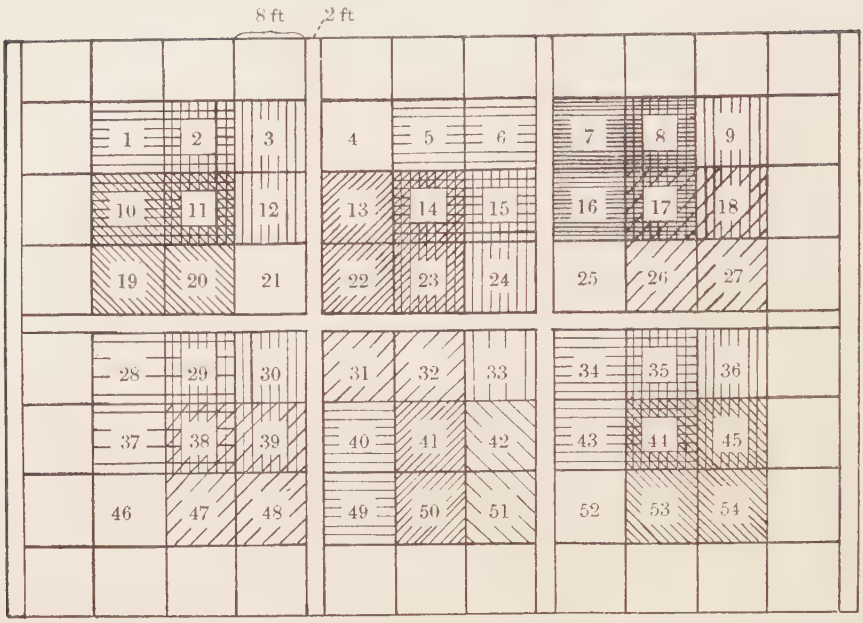


Fig. 1. Arrangement of plots in field trials at Ormskirk, 1922. Size of each plot = 8′ × 8′, each containing 40 plants. Width of paths = 2′. Control plots unshaded.

Plot 1. Formaldehyde, 0·19 %. Plot 2. Formaldehyde, 0·19 % + lime. Plot 3. Lime. Plot 4. Control. Plot 5. Formaldehyde, 0·02 %. Plot 6. Formaldehyde, 0·02 %. Plot 7. Paraformaldehyde, 0·05 %. Plot 8. Paraformaldehyde, 0·05 % + lime. Plot 9. Lime, 0·1 %. Plot 10. Formaldehyde, 0·19 % + dichlorcresol, 0·075 %. Plot 11. Formaldehyde, 0·19 % + dichlorcresol, 0·075 % + lime. Plot 12. Lime. Plot 13. Sulphur, 0·1 %. Plot 14. Sulphur, 0·1 % + lime, 0·1 % + formaldehyde, 0·02 %. Plot 15. Formaldehyde, 0·02 % + lime, 0·1 %. Plot 16. Paraformaldehyde, 0·05 %. Plot 17. Paraformaldehyde, 0·05 % + calcium polysulphide, 0·1 % + lime. Plot 18. Calcium polysulphide, 0·1 % + lime. Plot 19. Dichlorcresol, 0·075 %. Plot 20. Dichlorcresol, 0·075 %. Plot 21. Control. Plot 22. Sulphur, 0·1 %. Plot 23. Sulphur, 0·1 % + lime 0·1 %. Plot 24. Lime, 0·1 %. Plot 25. Control. Plot 26. Calcium polysulphide, 0·1 %. Plot 27. Calcium polysulphide, 0·1 %. Plot 28. Cheshunt compound. Plot 29. Cheshunt compound + lime. Plot 30. Lime, 0·1 %. Plot 31. Potassium polysulphide, 0·1 %. Plot 32. Potassium polysulphide, 0·1 %. Plot 33. Calnus, 300 gm. Plot 34. Formaldehyde, 0·01 %. Plot 35. Formaldehyde, 0·01 % + lime. Plot 36. Lime. Plot 37. Cheshunt compound. Plot 38. Cheshunt compound + ammonium polysulphide + lime, 0·1 %. Plot 39. Ammonium polysulphide + lime, 0·1 %. Plot 40. Formaldehyde, 0·02 %. Plot 41. Sulphur, 0·1 %. Plot 42. Spent oxides. Plot 43. Formaldehyde, 0·01 %. Plot 44. Formaldehyde, 0·01 % + dichlorcresol, 0·0375 % + lime. Plot 45. Dichlorcresol, 0·0375 % + lime. Plot 46. Control. Plot 47. Ammonium polysulphide. Plot 48. Ammonium polysulphide. Plot 49. Formaldehyde, 0·02 %. Plot 50. Sulphur, 0·1 %. Plot 51. Spent oxides. Plot 52. Control. Plot 53. Dichlorcresol, 0·0375 %. Plot 54. Dichlorcresol, 0·0375 %.

adopted in order to obtain the most thorough incorporation of the chemicals with the soil. This method was obviously impossible on a field-scale and yet the hypothesis on which we were working and which had been supported by our experience in the pot experiments demanded that a similar incorporation be achieved in the field if any success were to be expected. As already stated it is unlikely that ordinary agricultural implements such as ploughs, harrows, etc. could produce the intimate mixing required nor did hand-forking appear either a likely or indeed a possible method on a large scale. A method was finally suggested by one of us (W. A. R.) which was to utilise the Simar Rotary Tiller for this purpose. This machine is driven by petrol and a number of revolving tines tear up the soil in an extremely effective manner depositing it again a few inches away from its original position. The depth to which the tines pulverise the soil may be adjusted up to ten inches. The Piccard Pictet Company who are the makers and distributers of this machine very generously lent to us a 4 horse-power machine and helped us in every possible way to make the fullest use of it. The field tests during the years 1922–3–4 were carried out using the Simar Rotary Tiller as the implement of incorporation and whatever success has been achieved in our investigation is due in very large measure to its efficient working. The method of treatment was to spread the chemical as evenly as possible over the surface of the plot and then to cultivate the soil to a depth of 10 inches four, five or even six times lengthwise and crosswise. This number of cultivations was considered desirable since the efficiency of the treatment from our point of view was that of the least well-mixed portion and with a small machine of the type used by us it was difficult to avoid missing narrow strips. In most cases on the light sandy Ormskirk soil the chemical "disappeared" into the soil with one working. Thus when ground sulphur was spread on soil so thickly that the entire surface was bright yellow it was often difficult after the soil was worked over once with the Simar machine to detect any trace of the chemical with the naked eye. The Simar Rotary Tiller seems to have solved the problem of intimate incorporation of any finely divided substance or liquid with soil and gave us what we considered to be a satisfactory basis for our experimental work in the field.

Method of Estimation of the Disease. In previous Wart Disease research it has been usual to estimate the amount of disease present, and so in inverse ratio the efficacy of any particular fungicide, by separating the warted tubers from the clean tubers and then expressing the former as a percentage of the total crop. Although our pot experi-

ments had begun to make us doubt the value of this method it was retained for the field experiments of this year. Every plant was lifted separately and each tuber washed and examined minutely for the presence of Wart Disease. At the same time the exact position in the plot of each plant was noted and its freedom or otherwise from disease recorded. The warted tubers were separated from the clean ones and each portion weighed for each plot. Most treatments were represented by at least two plots.

The extensive experience gained in the lifting of this experimental crop showed that, as a method of estimating the amount of disease in the crop and thereby the relative efficiency of particular fungicidal treatments, the method described above (which had been used by other investigators in a still less accurate and controlled manner) was extremely unsatisfactory and liable to give a false impression of the actual facts.

For example, in examining the plots treated with different chemicals certain serious difficulties were encountered.

A striking feature of the experiments was the fact that the treatments acted differentially on the weed flora, certain plots being entirely free from weeds and others completely overrun. The competition reacted on the potato plants which in certain plots were stunted. The visible incidence of Wart Disease however appears to vary in direct ratio to the general growth and vigour of the host plant so that stunted or weakly plants are much less liable to show disease than large strongly growing plants. Thus the weed factor, which was very difficult to control owing to the great likelihood during the process of reintroducing *Synchytrium* sporangia on boots or hoes, very seriously interfered with the accuracy of the results.

Again, both host plants and warts developed and matured more rapidly under certain treatments than under others. This factor was not realised until too late and in any case the inconvenience and expense of conducting several liftings would have ruled out the possible remedy. All the plots had therefore to be lifted and examined at the same time and it was then found that whilst the warts in some plots were small and hard and obviously newly formed, in other plots they were large and old. In certain plots the warts had even "liquefied" and all that remained were a few vascular strands attached to the plant and a black stain in the soil. Thus in the latter case the percentage of warted crop measured by weight would be low whilst in the former, which might be a much less heavily infected crop, the percentage would be high.

An additional factor of critical importance arises in the life history of the parasite. The resting sporangia in the soil germinate and the swarm spores invade the eyes of the tubers or the meristematic points of underground or sometimes aerial shoots. Summer sori or further resting sporangia develop and the former dehisce, liberating large numbers of swarm spores which invade adjacent shoots or eyes in which numerous summer sporangia and later winter sporangia develop, thus spreading the disease. If by suitable treatment all the sporangia in the soil have been destroyed there is a complete absence of infection. If however the soil has not been successfully treated, or has been recontaminated after treatment, the sprouting tubers or plants are invaded at a number of points depending in large measure upon the number of resting sporangia surviving in the soil. Summer sori are rapidly formed and fresh infections follow. Thus from perhaps a very small beginning and to a large extent independent of the number of initial points of infection, the amount of disease increases sometimes with great rapidity. Given favourable weather conditions large warted growths are produced in a few weeks and thus the disease spreads. This continuous reinfection and spread by means of summer sporangia tends very quickly to mask the number of points of initial infection from the resting sporangia in the soil. It is, however, only the latter which is of any value in estimating the effect of fungicidal treatment of soil and, in order to obtain accurate results, it is imperative to lift the potatoes soon after the maximum amount of infection by resting sporangia in the soil has occurred and before this effect has been masked by the rapidly developing crops of summer sporangia. As noted above, however, the appearance and rapidity of development of warted tissues vary with the treatment; it occurs underground and is not easily visible and considerable supervision is required to choose the best time for the lifting of each plot. In our experimental work we could only hope to effect the best compromise.

Again, diseased plants showed a complete range from large tubers with perhaps a minute infection in one eye to tubers of various sizes which might be a mass of wart, often no trace of normal tuber being visible. In examining diseased material it was necessary either to separate the entirely clean tubers from those infected even in the slightest degree, the method adopted by us, or to cut off the warted growths; neither of which alternatives could give any accurate measure of the relative intensity of the disease.

Although therefore in the field experiments of 1922 the tubers were

all examined and the percentage of diseased tubers by weight recorded for each plot it was gradually forced upon us that results so obtained were often completely at variance with what were obviously the actual field results, and that, as a measure of the fungicidal efficiency of any particular chemical treatment such recording had little if any value and often might be misleading. This method of estimation and recording was abandoned after 1922 and although for that year a mass of data was obtained the quantitative results bore so little relation to the observed facts that it is not considered worth while to reproduce them here.

Results. During the lifting and detailed examination of the tubers certain definite effects began to emerge. The potato plants in control plots were well infected with Wart Disease. Clean or almost clean plots were obtained by treatment with 0·19 per cent. formaldehyde and with 0·1 per cent. sulphur. Where disease was present in these plots it was either very slight or situated at the edge of the plots. In the case of the 0·1 per cent. sulphur two double plots (13, 22–41, 50) situated in different parts of the field and each containing forty plants were quite free from disease. The same amount of sulphur applied as gasworks spent oxides and as potassium and calcium polysulphides reduced the amount of wart appreciably but not to the same degree as the pure sulphur. The above substances are placed in order of efficiency, the first having the greatest effect. In the plots treated with ammonium polysulphide containing the same amount of sulphur, there was no perceptible diminution in the amount of disease as compared with the control plots.

When the sulphur was used in conjunction with lime the effectiveness of the treatment was destroyed, but the combination of lime with the heavy dressing of formaldehyde did not reduce the efficiency of the latter.

The only other substance or mixture among those tested which produced any reduction of the disease was dichlorcresol; but this substance has an unpleasant smell which persists in the soil and on the tubers for more than six months and has a very bad effect on the potato crop. These disadvantages together with its high cost far more than outweigh its efficiency in eliminating the disease.

In addition to freeing the soil of sporangia, formaldehyde of a strength of 0·19 per cent. appeared to eliminate all other disease organisms and had a beneficial effect upon the crop. Furthermore it had the advantage of acting in the presence of lime. Since however a dose of ·02 per cent. formaldehyde was ineffective, the cost of eliminating the disease with

this substance from any considerable area would be prohibitive although for urgent use on a very small scale its application might be feasible.

A consideration of the results where various mixtures of chemicals had been applied showed no evidence that two or more substances used together had an effect greater than the sum of their effects when used separately. From the substances tested sulphur was selected for more extensive trial because it combined a high efficiency with a comparatively low cost. It was decided to concentrate on this one substance in the experiments of 1923.

Ormskirk, 1923.

The experiments of this year were planned as shown in Fig. 2, the aim being to test the effects of 5, 10, 15, 20, 30 and 40 cwts. per acre of sulphur respectively so that some idea of the minimum effective dressing might be obtained. The plots were in duplicate, one series being treated with ordinary ground sulphur and the other series with similar sulphur which had been inoculated with *Thiobacillus thiooxydans* (see p. 181). In addition there were two other plots (4, 12) which were treated with flowers of sulphur. The ends of all the plots received a heavy dressing of ground limestone—5 tons to the acre. Each plot contained 126 plants.

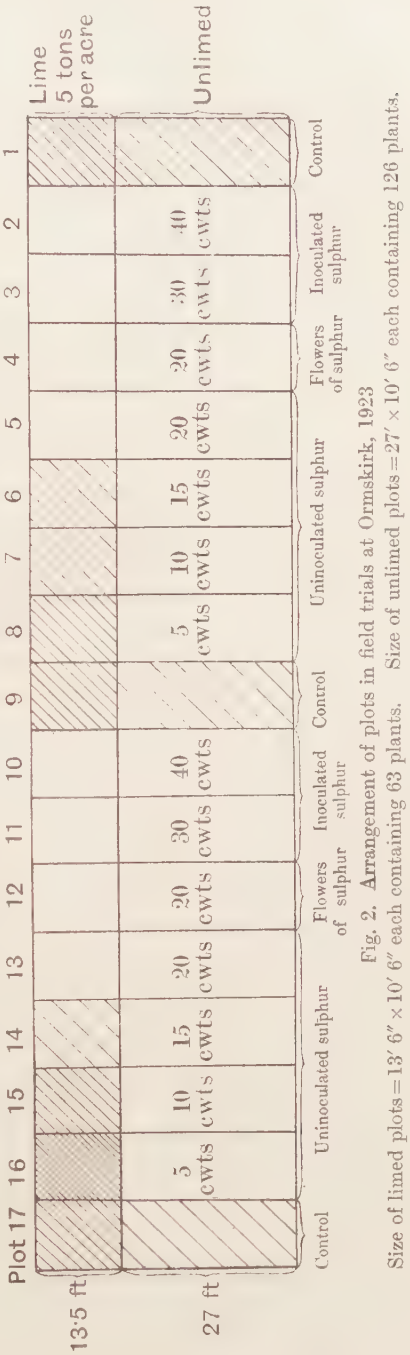


Fig. 2. Arrangement of plots in field trials at Ormskirk, 1923
Size of limed plots = 13′ 6″ × 10′ 6″ each containing 63 plants. Size of unlimed plots = 27′ × 10′ 6″ each containing 126 plants.

It had been intended in this year's trials to apply a new method of estimation of the amount of disease present (see pp. 165–166), but the season was so bad from the experimental point of view that this was not practicable. In 1923 the weather was such that Wart Disease only began to appear in any quantity some time after it had become necessary to lift our experimental crop. At the time of lifting only about one in six of our control plants were infected at all and those but slightly, whereas in the same field during 1924 when the weather favoured the appearance of the disease, 85 per cent. showed disease in varying degree but mostly with extremely heavy infection. The whole of the crop however in 1923 was lifted and examined and although because of the low percentage of infection in the control plots no final deductions could be drawn from these experiments, the results that were obtained were certainly suggestive.

The three control plots (1, 9, 17) were situated at the two ends and in the middle respectively and on careful examination it was found that the amount of infection though very small was uniform throughout. The entire series of plots treated with sulphur alone were free from disease except for one minute piece above ground on a stem near the edge of the plot. The limed ends of the plots receiving small amounts of sulphur, viz. 5, 10 and 15 cwts. per acre ground sulphur and inoculated sulphur (plots 6–8, 14–16) were definitely infected, the amount of disease decreasing as the amount of sulphur increased. Thus the addition of calcium carbonate seemed to reduce the effect of the sulphur. The presence of disease and its relative amount is shown in Fig. 2 by means of shading.

Thus whilst no final conclusions could be drawn the results of these experiments supported the general trend of evidence which indicated that sulphur was definitely toxic to the *Synchytrium* sporangia.

Ormskirk, 1924.

The 1924 experiments were designed to test more conclusively the indications obtained in the previous years. Since however inoculated sulphur had shown no improvement over ground sulphur at Ormskirk and was definitely less efficient at Hatfield (p. 177) in 1923 this form of sulphur was omitted. The experiments were carried out along the same general lines as in the previous season, the arrangement and treatment of one half of the plots being shown in Plate X. Each plot measured 10 ft. × 14 ft. and contained 140 plants. With the exception of plot 28, the spent oxides and sulphur were incorporated by means of the Simar

Rotary Tiller, but in the case of this one plot the sulphur was dug into the soil thoroughly with a fork in order to give a comparison of hand digging with Simar mixing. The machine incorporation of the chemicals was not carried out as thoroughly as we had intended, owing to the fall of heavy rain during the treatment which rendered further cultivation impossible owing to the condition of the soil. After treatment the plots were surrounded by a wind-break of coir-lewing as used in hop-fields. This was to prevent the re-contamination of the plots by wind-blown soil. This is a very serious difficulty since the Ormskirk soil, which is light and sandy, is easily blown by wind, and is probably more heavily contaminated with Wart Disease than any other soil in the world. It was also hoped that rabbits, hares, dogs etc. would be prevented from crossing the treated plots although of course the smaller vermin and birds (ground feeding sea-gulls and rooks abound in the region) would still have free access. Unfortunately in high gales the coir-lewing was blown down in places more than once, and some of the plots remained unprotected for varying periods of time.

Each plot contained ten rows of fourteen plants and the potatoes were set in three different series as shown below.

No. of plot	Date of treatment	Date of planting	Period between treatment and planting. Weeks	Date of lifting	Age of plants. Weeks
1–28	20–26. iii. 24	1–6. v. 24	6	19. viii. 24	15
43–56	20–26. iii. 24	11–13. vi. 24	12	½ plots 19. viii. 24 ½ plots 17. ix. 24	10 14
29–42	20–26. iii. 24	1. vii. 24	15	17. ix. 24	11

The system of deferred planting was adopted in order to ascertain the minimum period which should be allowed to elapse between the chemical treatment and the setting of the potatoes. The lifting was done in two parts in order to test the relation of age and growth of the plant to the length of time for which the treatment had been in operation and to distinguish if possible between "primary" infection from the soil and "secondary" infection of the same plant by means of summer sporangia from the primary warts (see p. 165).

Estimation of disease. In 1924 a great amount of disease was present and a new method of evaluation was adopted.

Each plant was lifted and examined separately and then given an

infection figure according to the amount of disease upon it—0 if absolutely free from disease; 1 if there were not more than two small warts upon it; 3 if practically the whole of the plant were warted, and 2 if the amount of infection was intermediate between 1 and 3. These infection figures, corresponding generally to the terms "clean," "light," "moderate" and "heavy," made no pretence at accuracy but were intended to express numerically the actual field result, *i.e.* the number of original points of infection by resting sporangia in the soil, which the previous "quantitative" methods had entirely failed to do. Every plant was examined personally by W.A.R. and W.B.B. and awarded its infection-mark, a test examination having shown that these two observers awarded identical values in practically every case. At the same time the exact position in the plot of each plant was recorded and notes were made of the position of the warts on the plant, whether entirely below the surface of the ground, below and at the surface, or on the stem and lower leaves above the ground. The distinctions of position are important, for the latter cases are probably to be interpreted as plants growing in sterile ground but secondarily infected through the agency of vermin, or wind-blown soil, especially in cases where there was no below ground infection, etc. as referred to on p. 165.

Sulphur. In Plate X B the degree of infection and the position in the first 28 plots of every plant are shown. The areas occupied by clean plants are left unshaded; those of plants with an infection mark of 1 receive one bar; those with an infection mark of 2 receive three bars, whilst those with an infection mark of 3 are completely black. The shading in the untreated plots (1, 5, 10, 14, 15, 19, 24) in this diagram shows that the whole experimental plot was thoroughly infected, and it is obvious that with increasing doses of sulphur the shading becomes lighter until plots 6 and 7 are almost clean.

These results may be interpreted quantitatively by expressing the sum of the infection marks for each plot as a percentage of the total possible, *i.e.* three times the number of plants in the plot. In Fig. 3 the percentage infection figures are plotted against the number of hundredweights of sulphur per acre applied. The calculated straight line of nearest fit to these values has been drawn ($1 = -6{\cdot}53\,S + 73$ where 1 = per cent. infection figure for an amount of sulphur S) and it will be seen that all the points referring to amounts of sulphur of less than 10 cwts. per acre lie quite close to this line. The straight line of nearest fit cuts the horizontal axis in the point corresponding to 11·2 cwts. of sulphur per acre, and it seems likely that if recontamination

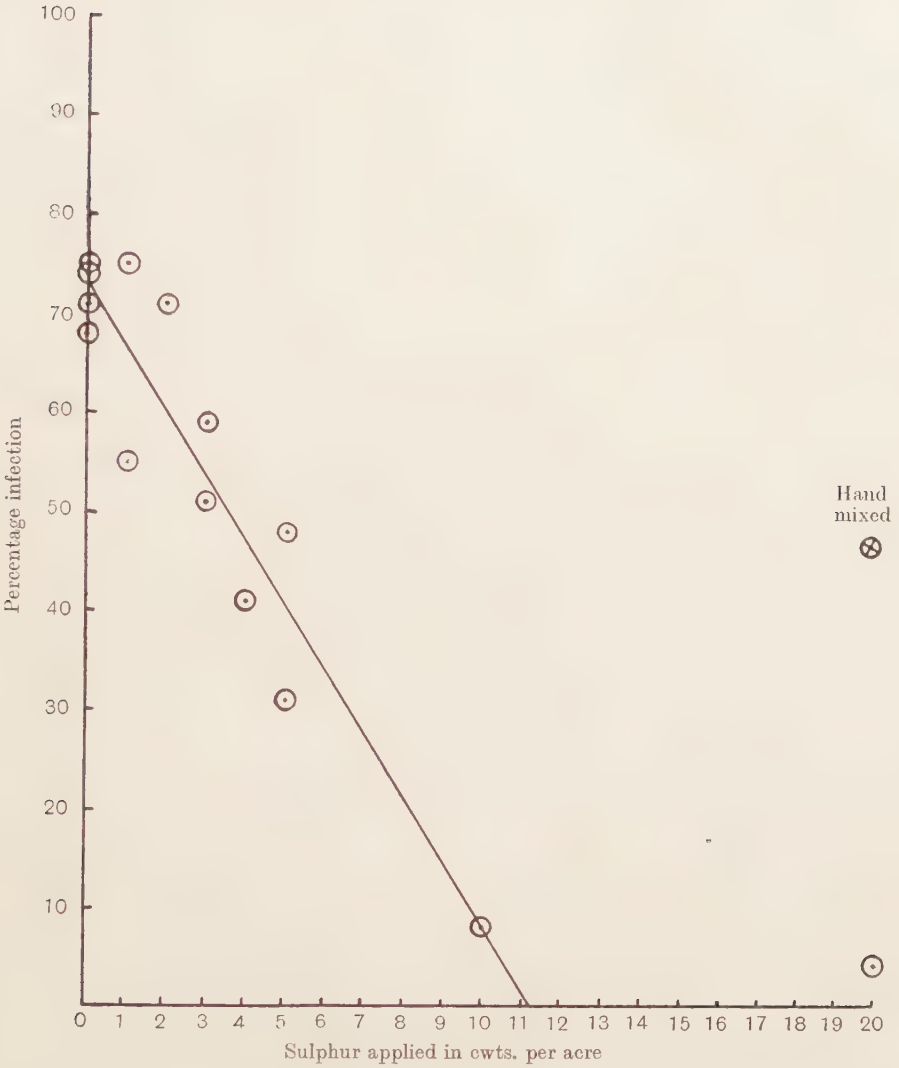


Fig. 3. Ormskirk, 1924, Plots 1–14. First planting. Ground-sulphur. Degree of infection by Wart Disease plotted against amount of sulphur applied.

from outside could be avoided this quantity of sulphur should be enough to free contaminated soil of *Synchytrium* sporangia.

If the above argument be correct the presence of nearly 4 per cent. of disease on plot 6, receiving 20 cwts. per acre of sulphur, needs explanation. On one side this plot abutted directly on to plot 5 which was untreated, and the infection of a few plants immediately adjacent to this

plot is likely to be the result of contamination carried from the untreated plot by wind, vermin and water splashes during heavy rain. If these plants are eliminated the infection figure is reduced to 2 per cent. which is made up of six spots of infection all situated at or above the surface of the soil. No disease was found in this plot below the surface of the soil, which suggests strongly that these small infections were due to sporangia carried by such extraneous agencies as wind-blown dust, vermin, birds, etc., against which, as we have already stated, the plots were only partially protected.

Plot 7, which received a dressing of 10 cwts. per acre of sulphur, has an infection figure of approximately 8 per cent. The greater part of this is made up of infection which is likely to have been carried in from the outside after the completion of the treatment at and above the surface of the soil, but in nine plants there was soil infection without any surface infection, which suggests that the soil itself is not quite free from the disease.

It has already been stated that, owing to the falling of heavy rain which rendered further cultivation of the soil impossible, the incorporation of the sulphur with the soil was done less thoroughly than had been our intention. The treatment however, in a year exceptionally favourable to the development of Wart Disease, had controlled this disease in the manner shown in Plate X B and Fig. 3. The question therefore arises whether the minimum quantity of sulphur necessary to effect the destruction of the *Synchytrium* sporangia could be reduced in amount by more thorough incorporation. This problem of the relation of dosage of sulphur to degree of incorporation is one which obviously will become of great importance if large areas are to be treated. The only definite evidence we have, throwing light on this relationship, is derived from plot 28. The treatment here consisted in spreading sulphur at the rate of 20 cwts. per acre evenly over the surface of the plot and then working it into the soil as thoroughly as possible by hand digging with a fork. With this amount of mixing the infection figure is reduced from about 70 per cent. the value for untreated soil to about 47 per cent., whereas the same dressing of sulphur incorporated with the soil by means of the Simar machine reduced the infection figure to 4 per cent. or less. The whole of our experience during the four years' trials recorded here strongly supports our original hypothesis that intimacy of mixture is imperative for any soil sterilisation work, and that extremely toxic substances may be relatively innocuous in the soil if badly mixed, whilst far less toxic substances may act as most efficient fungicides if thoroughly incorporated.

So far the discussion of the 1924 results has been confined to plots 1–14 which received varying dressings of sulphur, and there now remains to be considered the results obtained from plots 15–17 which received treatment with gasworks spent oxides.

Gasworks spent oxides. Spent oxides were tested for two reasons—firstly, as an alternative source of sulphur which might be obtained from any local gas-works with a consequent reduction or elimination of freight-charges and, secondly, because it was considered that the impurities in spent oxides might delay or even inhibit the action of micro-organisms in the soil which utilise sulphur as a source of energy and so change it into some sulphur compound which may be either more or less toxic than sulphur itself. The spent oxides were of two kinds, the one derived newly from the gasworks plant and the other having been weathered. Both were ground down to a powder but in neither case was the fineness of grinding comparable to that of the sulphur. The state of subdivision of the spent oxides was from the beginning recognised to be very unsatisfactory, but it was the best that could be obtained for the experiments and these local spent oxides were used for our treatments (plots 15–27). The results obtained are set out in Fig. 4. The positions of the points suggest a straight line relationship, but there is a much greater divergence from the straight line of nearest fit than in the case of ground sulphur (plots 1–14). The calculated amounts of the two spent oxides to give a clean result are 27 cwts. per acre of the weathered and 35 cwts. per acre of the unweathered. The greater divergence from the straight line and the increased amount of the minimum effective dressing are in accordance with what might be predicted from the unsatisfactory state of division of the spent oxides. It is possible that were the spent oxides used in as finely divided a condition as the ground sulphur they would be equally lethal to the *Synchytrium* sporangia when an equal weight of sulphur is applied in each case.

Plots 1–28 have been recorded in detail because they show what we believe to be the true result of the sulphur and spent oxides treatment. The plants on these particular plots were 15 weeks old when lifted, comparatively well-grown and the warts were fresh and vigorous and had not begun to rot away or to give rise to very much additional infection by summer sporangia.

The results of the remaining plots (29–56) are less satisfactory from a scientific point of view. The halves of plots 43–56 which were planted in June were lifted at the same time as plots 1–28 which were planted in May. Thus when examined the former were only 10 weeks old, while

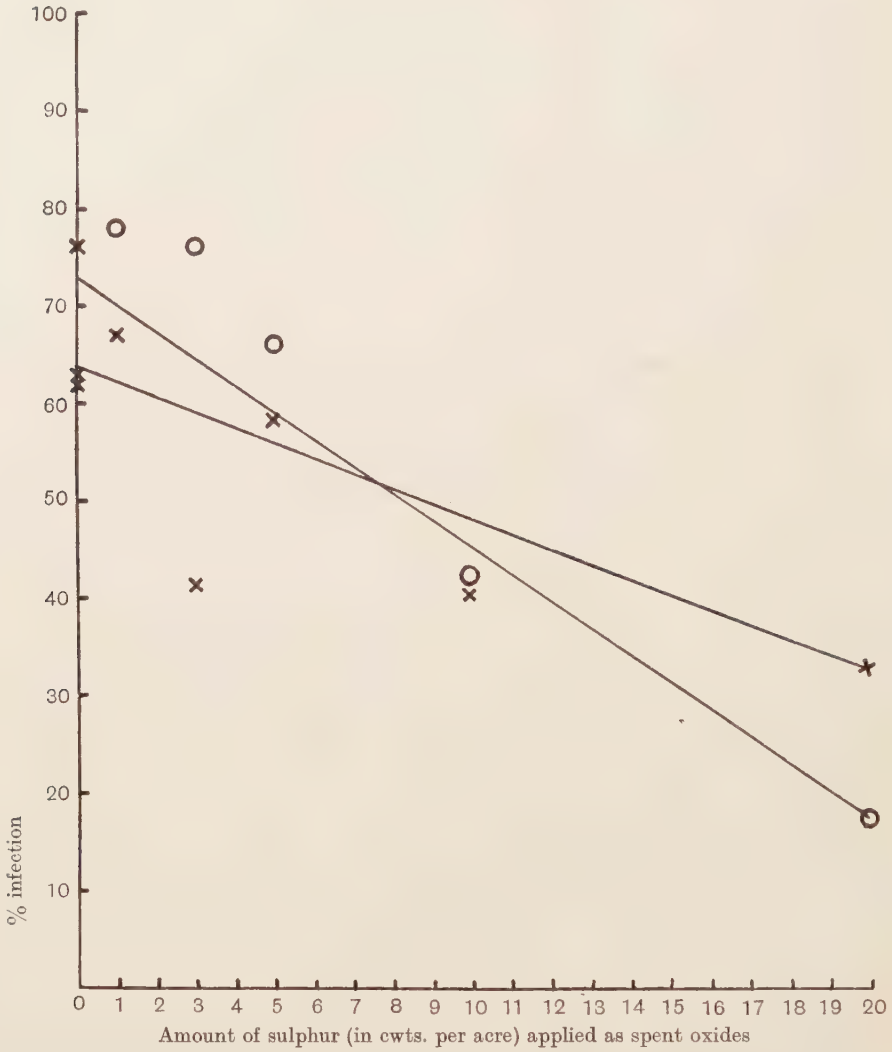


Fig. 4. Ormskirk, 1924. Plots 15–27. First planting. Spent oxides. Degree of infection by Wart Disease plotted against amount of sulphur applied as spent oxides.

the latter were 15 weeks old. Furthermore, owing to the very wet season, these later plantings had suffered from very severe weed competition which was very difficult to control, owing to the difficulties attendant on aseptic weeding. In consequence the plants on the halves of plots 43–56 were not well grown and the amount of infection was therefore small. Every plant however was lifted and examined and given an

infection mark. It was quite clear when these plants were lifted that they were too young to show the amount of disease that would have been present if they had been left in the soil for a few weeks longer. Although the actual "bulk" of diseased tissue was much less than in plots 1–28, the relative proportions in the several plots were very similar to those of the corresponding plots in the first series (plots 1–14). The results of the halves of plots 43–56 indicate that treatment with ground sulphur at the rate of about 11 cwt. per acre is sufficient to free the soil from contamination. Plot 44 receiving 20 cwts. per acre was entirely free from disease and plot 45 receiving 10 cwts. per acre had an infection figure of 1·5 per cent. whilst the control plots (43, 48, 52, 56) had infection figures of 66, 49, 38 and 79 per cent. respectively.

The remaining halves of plots 43–56 were lifted four weeks later on the 19th September. Although by this time the potato plants were much better grown, the greater part of the warts had become rotten and had disappeared as a liquid in the soil, making the award of infection figures almost impossible. Further, owing to the lengthy period during which the plants had been infected and to the liquefaction of the warts, the amount of infection by summer sporangia was great and tended to eclipse the initial infection from sporangia in the soil. When the results are plotted they can, therefore, be less easily represented by a straight line. The plot receiving 20 cwts. of sulphur per acre was clean, save for a few plants in the outside row in contact with untreated soil. The plot receiving 10 cwts. per acre had however an abnormally high infection figure, but we consider this to be due to recontamination of the plot during one of the unprotected periods when the lewing was blown down, followed by the intensifying and local spread of the disease by summer sporangia. Making allowances for this aberrant plot, the results point to the same general conclusion as the previous ones.

The plants in plots 29–42 had only been set in July. By the middle of September when it was necessary to lift them they had made little growth and the amount of disease present was so slight as to make it impossible to derive any sound results from them. Two sample rows only from the middle of each plot were lifted and examined, but no infection figures could be given and the plots were discarded.

Generally reviewing the Ormskirk experiments of 1924 in the light of our past experience, certain deductions may be drawn. Firstly no system of recording gives an accurate picture of the results as observed in the field although the method adopted of awarding infection marks approaches this ideal most nearly. Secondly, infection by means of

summer sporangia with its subsequent developments may mask the number of original points of infections due to resting sporangia surviving in treated soil, and it is only the latter which gives the fungicidal value of any particular soil treatment. Thirdly, ground sulphur applied at a rate greater than 10 cwts. and less than 20 cwts. per acre and incorporated with the soil by means of a Simar Rotary Tiller, in a season of exceptionally heavy wart, almost, if not quite, eliminated the disease on the Ormskirk soil.

Experiments carried out at Hatfield.

By the courtesy of His Grace the Marquis of Salisbury we were able to carry out a few experiments on a small naturally infected area isolated from all other cultivated land and situated in the middle of Hatfield

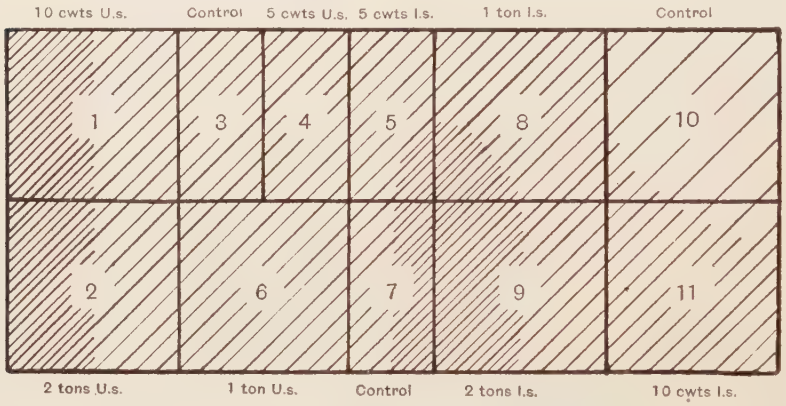


Fig. 5. Hatfield, 1922 and 1923. Approximate degree of infection by Wart Disease before treatment shown by intensity of shading. The position of the plots of 1923 is shown together with the treatment, the sulphur being shown in cwts. pr acre.

Park. The soil of this garden is a heavy clay with flints and contains little or no reserve of calcium carbonate. Slight differences are visible between the soil at the two ends of the plot.

1922 *Experiments.* Beyond the fact that diseased tubers had been reported from this garden, nothing was known about the distribution of the fungus in the soil or the degree to which it was present. Hence it was necessary to investigate these two points before any fungicidal experiments could be carried out on the plot. In 1922 therefore the land was merely planted with a susceptible variety and the plants at the close of the season lifted, examined, and the presence or absence and relative incidence of disease recorded. From these observations Fig. 5 was prepared, which shows by the intensity of shading the

initial distribution of the disease. The areas marked as heavily contaminated are, though not quite so heavily contaminated as the Ormskirk plots, quite comparable thereto. After the plants had been lifted an attempt was made to equalise the degree of contamination over the whole area under experimentation. Plot 10 was not included in this latter treatment.

1923 *Experiments.* The experiments at Hatfield were carried out in substantially the same way as at Ormskirk (p. 168) except that the irregularities in the distribution of contamination mapped out in 1922 necessitated more careful arrangement to provide adequate "control" plots and to allow of fair comparison being made between the various treatments tested. The arrangement of the plots is shown in Fig. 5 and Plate XI A. Attention was concentrated on the testing of ground sulphur and the same inoculated with *Thiobacillus thiooxydans*. The treatment was carried out immediately after the completion of the work at Ormskirk in the spring, the sulphur being thoroughly incorporated with the soil by means of the Simar Rotary Tiller. The potatoes were set a few days after the cultivation was finished.

At the close of the season every plant was lifted and examined, its exact position in the plot recorded and a value put upon its degree of infection according to the scale—clean, very light, light, moderate, heavy and very heavy. This method of evaluating the disease was obviously arbitrary, but on being tested it appeared to give a sufficiently accurate picture of the field results for our purposes. Plate XI B was constructed from the field notes. Each plant is looked upon as occupying a rectangle, and this is shaded according to the degree of infection present.

It was quite obvious at lifting that uninoculated sulphur applied to plot 2 at a rate of 2 tons per acre had brought about a very marked diminution in the amount of contamination. This result is made the more convincing if Fig. 5, mapping the original distribution of contamination, be compared with Plate XI B showing the 1923 results. The way in which the amount of disease suddenly decreases in passing from the control plots 3 and 7 to plot 6, which received a dressing of 1 ton of uninoculated sulphur per acre, establishes the fact that this treatment, although less effective than 2 tons per acre, caused an appreciable lessening in the degree of infection. On the other hand, treatment with 5 cwts. and 10 cwts. respectively had practically no effect upon the incidence of the disease.

The results obtained by treatment with inoculated sulphur were strikingly different. Plot 9, which received a dressing of 2 tons, inoculated

sulphur per acre contained practically as much disease as plots 3 and 7 which received no treatment of any kind. There is in fact no indication that inoculated sulphur has any influence in reducing the amount of disease on this type of soil. Perhaps this difference between the actions of the two kinds of sulphur is best seen by comparing plots 2 and 9 in which the original distribution of contamination was very similar and which, but for the treatment, would have shown approximately equal amounts of disease.

The results are represented in graphical form in Fig. 6. In each plot the total number of infected plants irrespective of the degree of infection is represented as a percentage of the total number of plants in the plot. It is seen that as the quantity of uninoculated sulphur increases there is in general a marked increase in the percentage of clean plants. In the case of the inoculated sulphur no such effect is visible, such fluctuations as do occur being attributable to irregularities in the initial contamination.

It was unfortunate in these experiments that the potatoes could not be lifted before a considerable amount of secondary infection by summer sporangia and rotting of warted tissues had occurred.

1924 *Experiments.* Owing to the small area of land at our disposal it was necessary this year to use previously treated plots for our experiments. This fact, added to the very unequal distribution of contamination due to the 1923 work and the excessive rains of 1924 which made the working and weeding of the heavy clay soil very difficult and "secondary" infection and rotting of warted tissues almost impossible to cope with, caused the experiments of 1924 to have little value. Only one result is worth reporting here. Plot 1 (Plate XI), which in 1923 received 10 cwts. of uninoculated sulphur per acre, was divided into two plots in 1924. One half was left untreated and the other half was treated with two tons of uninoculated sulphur per acre. The latter half was quite free from disease, whilst the control half, as in the 1923 experiments, was very heavily infected.

Reviewing generally the work at Hatfield, there seems little doubt that ground sulphur incorporated with the soil by means of a Simar Rotary Tiller is an efficient fungicide against Wart Disease of potatoes. On the heavy clay soil, the amount of sulphur required is more than double that necessary to achieve a similar result on the light soil of Ormskirk.

SOIL REACTION IN RELATION TO SULPHUR TREATMENT.

It has been shown in the preceding sections that sulphur acts as an efficient fungicide towards Wart Disease if thoroughly incorporated with infected soil in the field.

It is a well-known fact that sulphur is rapidly oxidised in the soil to sulphuric acid, which gives to the soil an acid reaction unless ample reserves of calcium carbonate are present. Attempts have been made, especially in America, to correlate the degree of infection of potatoes by scab [*Actinomyces* (*Oospora*) *scabies*] with the soil reaction; and it has been suggested that the fungicidal action of sulphur in this case is to be explained simply by the increase in the hydrogen ion concentration of the soil. It thus became of interest to ascertain whether the same explanation might possibly hold in the eradication of Wart Disease by sulphur. The question has a considerable practical significance, for the amounts of sulphur needed to produce a defined degree of acidity can be estimated from the titration curve for the soil. It would follow that soils containing appreciable amounts of calcium carbonate would require very heavy dressings of sulphur to produce the necessary reaction, and that customary dressings would be of no value. Such heavy dressings are likely to affect adversely the growth of succeeding crops. If however the sulphur acts independently of the degree of acidity produced, it might easily become possible to eliminate Wart Disease, without rendering the soil sufficiently acid to cause such damage.

In planning the field experiments it was possible in certain cases to arrange the treatments in such a way as to throw some light on this problem; but this could only be done so long as such an arrangement did not interfere with the main purpose of the investigation, viz. the elimination of Wart Disease from contaminated land. Throughout the experiments measurements of the soil reaction were made on samples taken after the removal of the crop from all those plots which might give information on the subject. Though of necessity these observations are incomplete, they seem sufficiently suggestive to justify their inclusion in the paper.

The soil reaction (*p*H value) was determined by the hydrogen electrode method, using the technique described by Crowther elsewhere (1). The measurements were made on suspensions prepared by shaking 20 gm. of air-dried soil in 100 c.c. of water for one hour in an end-over-end shaker. The results of each series of experiments are discussed separately.

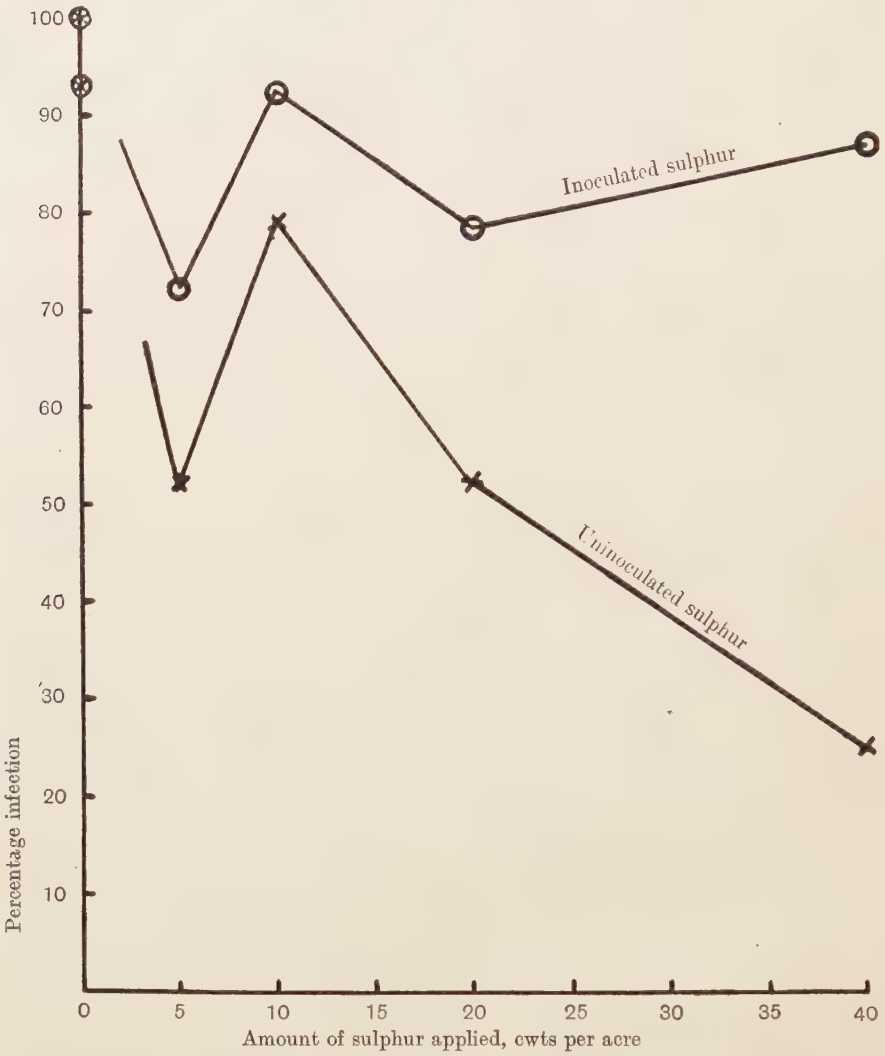


Fig. 6. Hatfield, 1923. Comparison of inoculated with uninoculated sulphur.

Ormskirk, 1922 (2).

In Table III are given the *p*H values and the degrees of infection by Wart Disease for a series of plots receiving equal amounts of sulphur (1 ton per acre) in various forms, viz. as ground sulphur, as the polysulphides of ammonium, calcium, and potassium and as spent oxides respectively.

Table III.

Treatment	*p*H	Reduction of Wart Disease
Untreated	5·3	—
Sulphur and lime (1 ton per acre)	3·8	Nil
Ammonium polysulphide	5·5	Nil
Calcium polysulphide	5·00	Slight
Spent oxides	5·3	Considerable
Potassium polysulphide	4·3	Considerable
Sulphur	3·6	Complete

Ground sulphur and potassium polysulphide caused considerable increases in the acidity of the soil and very striking reductions in the amount of Wart Disease; calcium polysulphide had less marked but similar effects, but ammonium polysulphide had no significant effect on either the reaction or the amount of disease. These facts suggest a possible correlation between increase in acidity and control of the disease. On the other hand, the spent oxides caused a marked reduction in the amount of disease without having any appreciable effect on the acidity of the soil, whilst the addition of lime only slightly reduced the acidity produced by ground sulphur but completely destroyed its fungicidal properties, suggesting that elimination of the disease is not necessarily dependent on high soil acidity. The high efficiency of formaldehyde in controlling Wart Disease was not affected by the addition of lime.

Ormskirk, 1923.

Lipman(7) and his co-workers in America have shown that the oxidation of sulphur in soil is due largely to biological agencies. Since some soils appear to be comparatively poorly provided with the necessary organisms, they have recommended the inoculation of ground sulphur with *Thiobacillus thiooxidans*. Martin(9) found that such inoculated sulphur was more efficient than uninoculated sulphur in controlling scab [*Actinomyces* (*Oospora*) *scabies*] in potatoes. The experiments were therefore duplicated in 1923 so as to secure a comparison of uninoculated with inoculated sulphur, both alone and with the addition of calcium carbonate at the rate of 5 tons per acre. The final *p*H values for both series are plotted in Fig. 7 against the amounts of sulphur applied.

As can be seen from this diagram, the points lie scattered about two "titration curves," and there is no consistent difference between the efficiencies of the inoculated and uninoculated forms of sulphur, in raising the acidity of the soil. No explanation can be offered of the anomalous results obtained with the heaviest dressings of sulphur (2 tons

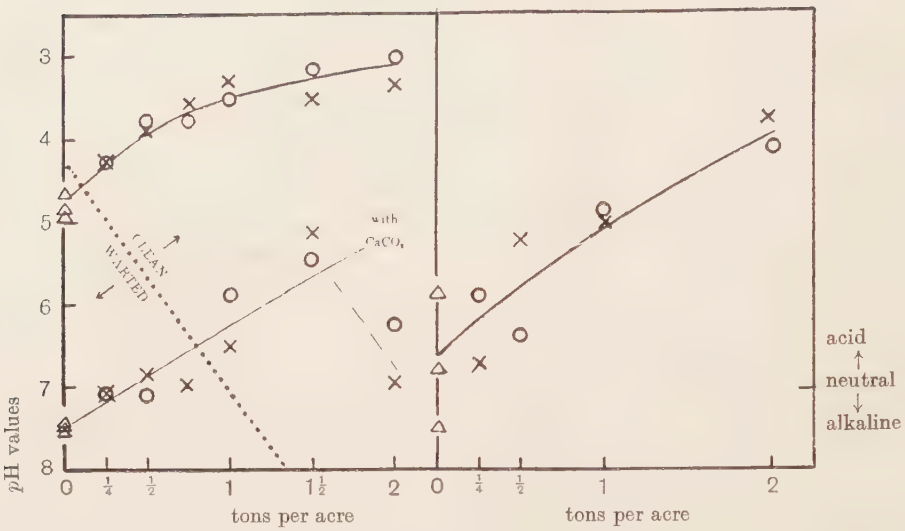


Fig 7. Fig. 8.

*p*H values of Ormskirk soil (Fig. 7) and Hatfield soil (Fig. 8) after various dressings of sulphur.

△ Untreated. ⊙ Inoculated sulphur. × Uninoculated sulphur.

per acre) in the presence of lime. Within the limits of this experiment, no difference was discovered in the field between the efficiencies of inoculated and uninoculated sulphur in controlling Wart Disease. The dotted line in Fig. 9 separates the points representing the plots infected with Wart Disease from those representing the clean plots. It will be seen that the clean "limed" plots were all less acid than the three untreated plots, each of which was infected. There is thus no critical acidity determining the elimination of the disease, for if there were this dotted line should lie parallel to the horizontal axis.

Hatfield, 1923.

These experiments were made on a heavy clay soil, which proved to be irregular (see p. 176). The wide variations in reaction of the three untreated plots and of those with small dressings of sulphur may be seen in Fig. 8. The plots with the two heavier dressings (1 and 2 tons of sulphur per acre) which are of the greatest interest in the present connection, however, show no appreciable difference in acidity between the plots dressed with inoculated and uninoculated sulphurs respectively. All of the plots were heavily infected with Scab [*Actinomyces* (*Oospora*) *scabies*] as well as with Wart Disease; observations were therefore made

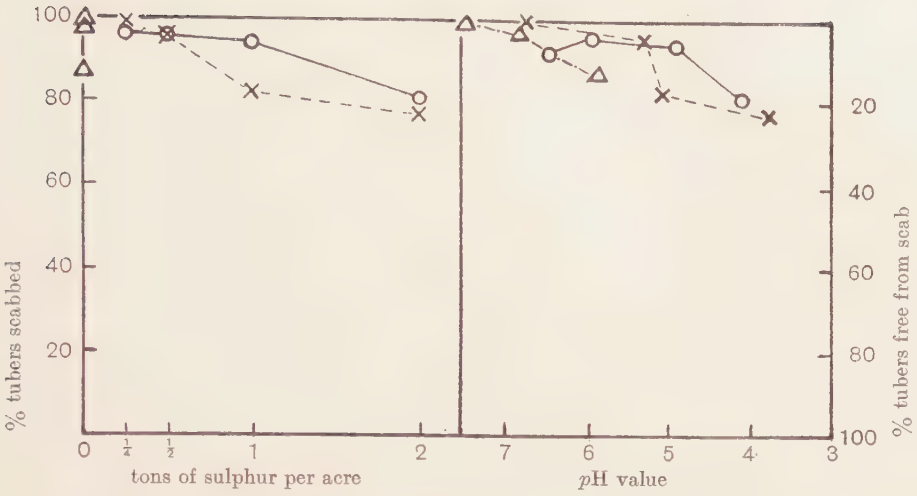


Fig. 9 Fig. 10

Hatfield. Infestation by common Scab as a function of amount of sulphur applied (Fig. 9) and of final *p*H value of soil (Fig. 10).
△ No sulphur. ⊙ Inoculated sulphur. × Uninoculated sulphur.

in regard to the distribution of both diseases in relation to the acidity of the plots.

Figs. 9 and 10 show the degree of infestation by Scab plotted against the amount of sulphur applied, and the final *p*H values respectively. The heavier dressings of sulphur are seen to have reduced the amount of Scab, and there is some evidence that the uninoculated sulphur was somewhat more efficient than the inoculated sulphur. Little significance can be attached to this latter result however, since it is possible that the heavier dressings of sulphur may have caused a certain amount of "burning" of the tubers; no method presented itself in the field at the time for differentiating simply between this "burning" and "scabbing." Subsequent microscopic examination in the laboratory of tubers from the plots with the heaviest dressings of sulphur made it clear that most of the diseased appearances were due to Scab [*Actinomyces* (*Oospora*) *scabies*] but it is possible that the values indicated in Figs. 9 and 10 are rather too high, especially for the heaviest dressings. In Fig. 10 there is a slight correlation between increasing acidity and decreasing scabbing, especially in the untreated plots. There is no evidence however of any limiting *p*H value such as was found by certain American investigators. On this soil heavy infection occurred with an acidity as high as *p*H 4·0.

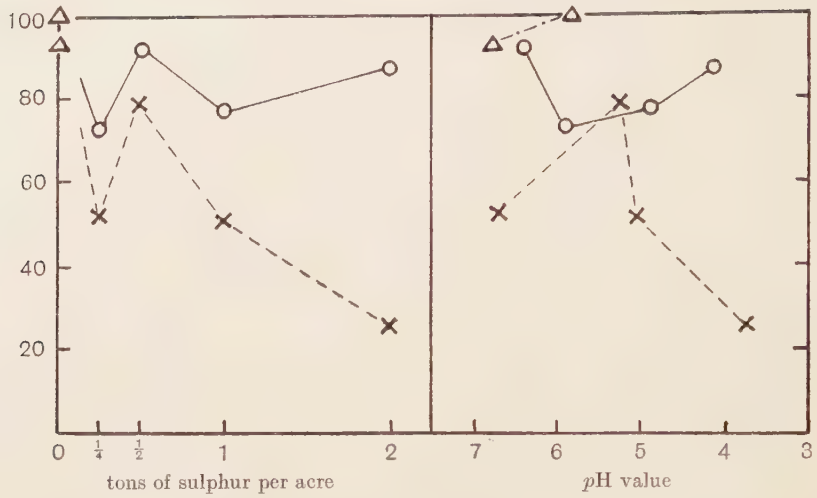


Fig. 11. Fig. 12.

Hatfield. Infestation by Wart Disease as a function of amount of sulphur applied (Fig. 11) and of final *p*H value (Fig. 12)

△ No sulphur. ⊙ Inoculated sulphur. × Uninoculated sulphur.

The results for Wart Disease are presented in the same manner in Figs. 11 and 12. In view of irregularities in the original distribution of infection (see Fig. 5) no precise significance can be attached to the form of these curves. A fairly reliable comparison may be made however between pairs of corresponding points (*i.e.* for plots dressed with equal weights of inoculated and uninoculated sulphur respectively), since these were distributed in the field so as to secure approximately equal initial infections.

The fact that in each pair of corresponding plots the uninoculated sulphur gave a much cleaner crop than the inoculated is quite strong evidence that the inoculated sample was less effective than the uninoculated one in eliminating Wart Disease. The difference is especially marked for the heaviest dressings, where it has already been shown that the soil reactions and the effects on Scab are approximately equal. The plot receiving 2 tons of uninoculated sulphur could be readily picked out from the surrounding plots on a superficial examination, and, as may be seen in Plate XI, the edges of this plot were marked by sharp changes in the degree of infection. There was no correlation between the amount of infection by Wart Disease and the final reaction of the soils (Fig. 12).

A crucial laboratory experiment was planned with a view to differ-

entiating between the effects of the addition of sulphur in increasing soil acidity and its effects in other directions. A series of equivalent amounts of sulphur, of the same order as the field dressings, was put up in bottles of Ormskirk soil at a suitable moisture content. A comparison was made between (1) sulphur alone, (2) sulphur + an equivalent amount of calcium carbonate, (3) sulphur + excess of calcium carbonate and (4) equivalent amounts of sulphuric acid. After six weeks the soil was transferred to pots and potatoes were planted. Unfortunately the experiments failed since no infestation by Wart Disease took place even in the control pots. The experiment is being repeated. The laboratory measurements of the soil reaction are however of some interest.

The *p*H values after six weeks' incubation are given in Table IV for one such group of experiments.

Table IV.

pH values of soils incubated for six weeks (17·8 *per cent. water*).

	Sulphur (or equivalent added) as % dry soil						
Treatment	0	(1) ·027	(2) ·054	(3) ·080	(4) ·107	(5) ·161	(6) ·214
I. Sulphur	4·29	3·81	3·63	3·49	3·40	3·13	3·09
II. Sulphuric acid	—	3·88	3·68	3·50	3·38	3·20	3·09
III. Sulphur + equiv. calcium carbonate	—	4·03	4·05	3·99	3·90	4·00	3·90
IV. Sulphur + 2 % calcium carbonate	—	7·44	—	—	—	—	7·42

Sulphur gave *p*H values almost identical with those given by equivalent amounts of sulphuric acid (II). Series III shows that it is possible to produce a series of soils in which the oxidation of various amounts of sulphur has taken place without any considerable differences in the final reactions. No change from the initial reaction occurred in the presence of excess of calcium carbonate (IV).

In a second group of similar experiments bottles were opened for *p*H measurements at frequent intervals in order to ascertain the time needed for complete oxidation. These results are presented in Fig. 13. Sulphur alone gave no change in reaction after five days but a marked change in 14 days, when the *p*H values were in the order of the amounts of sulphur applied. Further changes proceeded slowly up to six weeks. The sulphur in the smallest dressings appeared to oxidise more slowly than that in the heaviest ones. In this group of experiments sulphur gave appreciably lower acidities than equivalent amounts of sulphuric

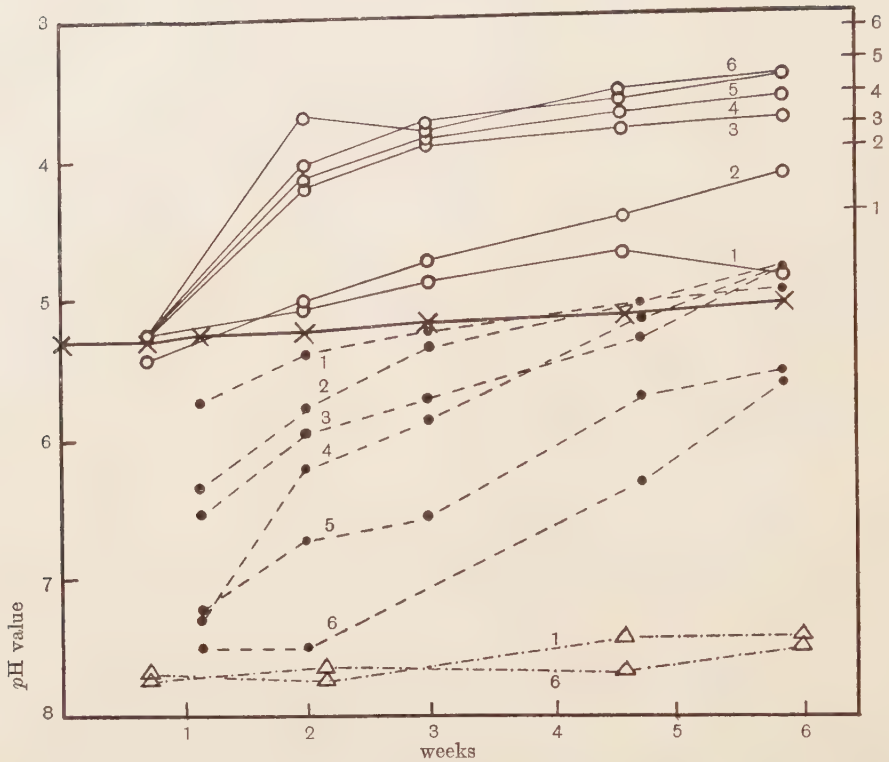


Fig. 13. Change of *p*H value of soils treated with sulphur

Series —	×	no sulphur	1 = ·027 % sulphur or equivalent
I	○	sulphur	2 = ·054 ,, ,,
III	●	sulphur + equivalent $CaCO_3$	3 = ·080 ,, ,,
IV	△	sulphur + excess $CaCO_3$	4 = ·107 ,, ,,
II	–●–	sulphuric acid (final values only)	5 = ·161 ,, ,,
			6 = ·214 ,, ,,

acid. Interaction of the soil with calcium carbonate, either in the bottles or during the preparation of the suspension, took place very rapidly; the oxidation of sulphur was appreciably slower than in the more acid soils, without calcium carbonate.

Hence, it is seen that a minimum interval of three weeks should be allowed to elapse between the application of the sulphur and the planting of the potatoes to allow the sulphur to have its full fungicidal effect. Owing to the exigencies of time and place it was not possible in many of our field experiments to allow this period to elapse before planting.

Effect of Sulphur treatment on Crop.

In the work that has been recorded our attention was focussed on the effect of the treatments on the amount of Wart Disease affecting plants growing in previously contaminated land and it was not possible to give more than a cursory glance at the effect of the several treatments tested upon the plant itself as measured by the weight of crop produced. Where treatments such as dichlorcresol had an obviously bad effect on the crop more than sufficient to compensate for their fungicidal effect upon the parasite they were abandoned.

In the case of sulphur the smaller rates of application produced plants fully equal to the control plants but doses of anything more than 10 cwts.—15 cwts. per acre had an adverse effect on the growth of the plants. This however only showed when the potatoes were planted immediately after treatment and the longer the period between treatment and planting the less deleterious was the effect of the sulphur upon the crop. From our field experience it seemed fairly clear that sulphur incorporated in the autumn or early winter should produce its full fungicidal effect and be oxidised long before the sets are planted in March. Owing to the mechanical difficulties and extra expense of such winter treatment it was impossible to carry it out in 1923 and 1924 but experiments are now under way whereby autumn treatment, spring treatment, and combined spring and autumn treatments are being tested.

Conclusion.

Our experiments during the years 1920–4 and more particularly those of last year seem to show that it is possible to destroy the resting sporangia of *Synchytrium endobioticum* in soil intensely contaminated with this organism. On the light sandy soil at Ormskirk it is possible, after treatment with about 12 cwts. or more of ground sulphur to the acre, to cultivate varieties of potato very susceptible to Wart Disease on contaminated land and to obtain a clean crop. A vital feature of the treatment is thorough incorporation of the sulphur with the soil and this appears to be successfully achieved by means of the Simar Rotary Tiller. On the heavy clay soil at Hatfield it is possible to destroy the fungus but a dressing of at least two tons of sulphur per acre is required.

On a large field scale the application of the above amounts of sulphur is not yet practicable but in the treatment of small and isolated outbreaks in the middle of large areas devoted to the cultivation of potatoes it would seem to be economically possible.

The present paper is essentially of the nature of a progress report and although there are numerous questions of theoretical and practical importance intrinsic in the work the amount of reliable evidence is as yet so meagre as to make any discussion of these unprofitable.

Summary.

As susceptible varieties of potato are still widely cultivated and sporadic outbreaks of Wart Disease are a serious menace, it is imperative to find a method whereby the winter sporangia of *Synchytrium endobioticum* in contaminated soil may be killed. Previous studies and the unusual difficulties presented by the problem are discussed. Results of experiments extending over four years are recorded.

During 1920–2 pot experiments were carried out to test various chemicals alone and in conjunction with steam. Steaming the soil proved effective, but offered little hope of being economically possible as a field treatment. The amount of disease was reduced by sulphur, calcium and potassium polysulphides, formaldehyde, dichlorcresol, chlordinitrobenzene and nitrobenzene. Satisfactory infection was not obtained in pot experiments; this method was therefore abandoned in favour of field experiments.

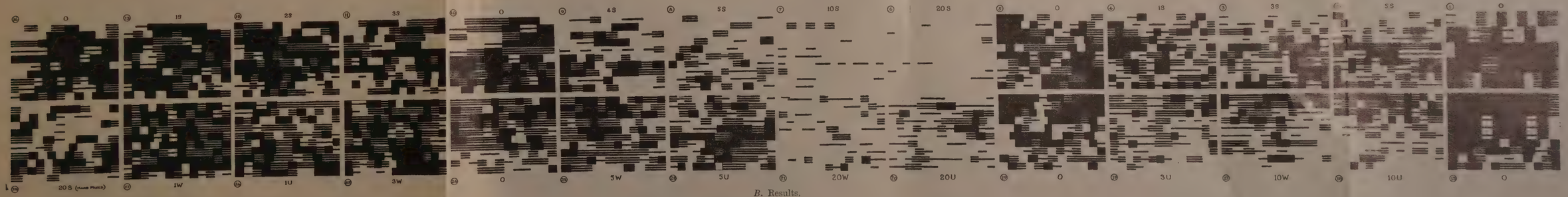
The incorporation of chemicals with the soil in the field was carried out with the Simar Rotary Tiller, great care being taken to ensure very thorough and even distribution. Results suggest that the efficiency of the treatment depends on this thoroughness of incorporation. During 1922 a selection of the chemicals tried in 1921 and others were tested. From these sulphur was selected in 1923 for more extensive study as being the most hopeful, because of its efficiency and cheapness.

In 1924, a year of very heavy disease, it was proved at Ormskirk that when the dose of ground sulphur was increased through 1, 2, 3, 4, 5, 10 cwts. per acre the degree of infection was reduced in direct ratio from 73 per cent., the value for untreated soil, to 8 per cent. for an application of 10 cwts. per acre. Doses greater than the latter did not completely eradicate the disease; but there are reasons for thinking that the small amount of disease in certain of the plots was due to recontamination of those plots later in the season. When the results are represented in graphical form the straight line of nearest fit to the experimental values cuts the horizontal axis at a point representing 11·2 cwts. per acre of sulphur; and, in the absence of recontamination, this quantity of sulphur should be slightly more than the minimum necessary to free the Ormskirk soil of disease.

Ormskirk 1924.

14	13	12	11	10	9	8	7	6	5	4	3	2	1
Control.	1 cwt. sulphur	2 cwts. sulphur	3 cwts. sulphur	Control	4 cwts. sulphur	5 cwts. sulphur	10 cwts. sulphur	20 cwts. sulphur	Control	1 cwt. sulphur	3 cwts sulphur	[illegible] cwts. sulphur	Control
20 cwts. sulphur hand mixed	1 cwt. spent oxides weathered	1 cwt. spent oxides not weathered	3 cwts. spent oxides weathered	Control	5 cwts. spent oxides weathered	5 cwts. spent oxides not weathered	20 cwts. spent oxides weathered	20 cwts. spent oxides not weathered	Control	3 cwts. spent oxides not weathered	10 cwts. spent oxides weathered	10 cwts. spent oxides not weathered	Control
28	27	26	25	24	23	22	21	20	19	18	17	16	15

A. Key to arrangement and treatment of plots. Plots 1–28 only shewn. Size of plots = 10′ × 14′ each containing 140 plants. Quantities given as cwts. per acre.



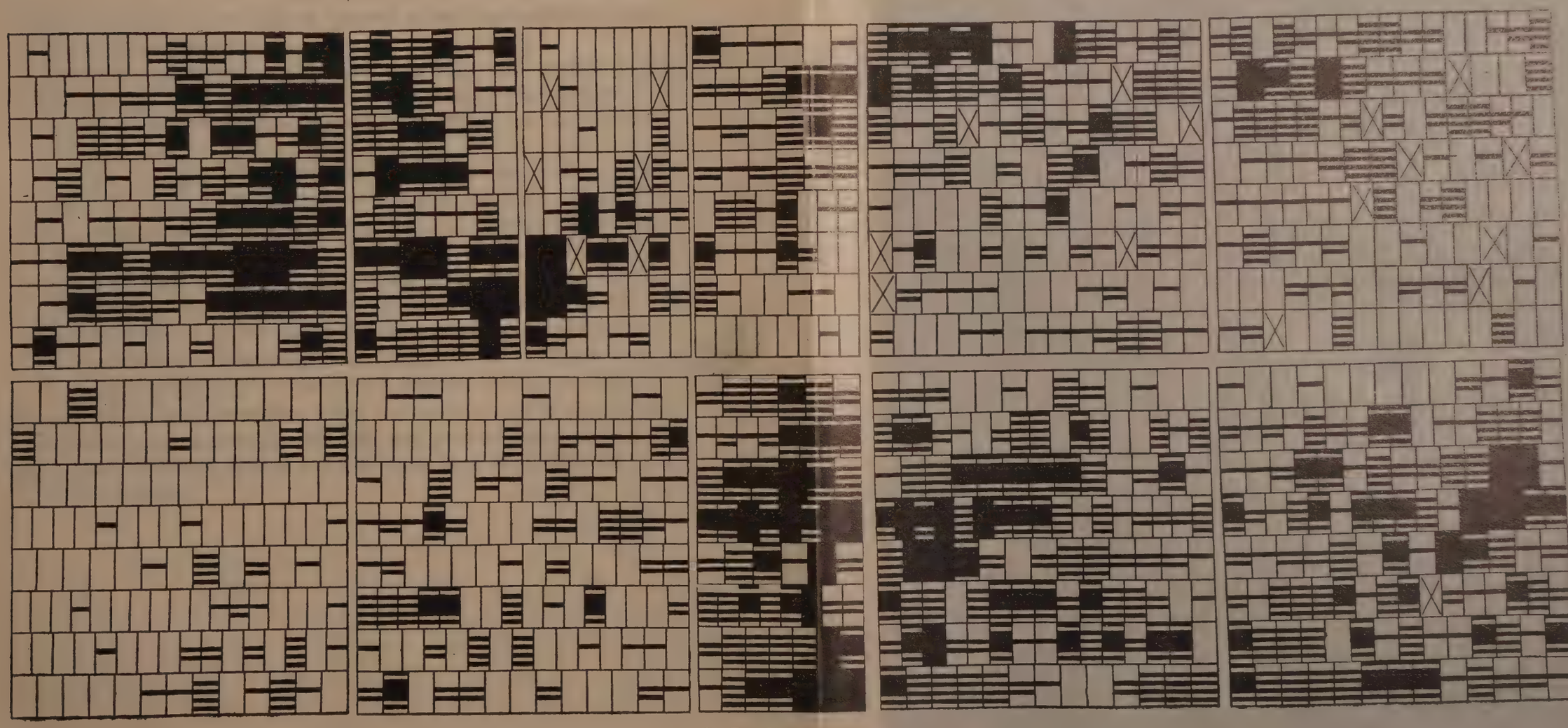
B. Results.

Degrees of intensity of infection — clean — light — moderate — heavy

Hatfield, 1923.

1	3	4	5	8	10
10 cwts. uninoculated sulphur	Control	5 cwts. inoculated sulphur	5 cwts. inoculated sulphur	20 cwts. inoculated sulphur	Control
40 cwts. uninoculated sulphur	20 cwts. uninoculated sulphur		Control	40 cwts. inoculated sulphur	10 cwts. inoculated sulphur
2	6		7	9	11

A. Key to arrangement and treatment of plots. Amount of sulphur in cwts. per acre.



B. Results.

Degrees of intensity of infection

clean' very light light moderate heavy very heavy

On the heavy clay soil at Hatfield it was found necessary to use much heavier applications of sulphur (about 40 cwts. per acre) to ensure absolutely clean plots.

Gasworks spent oxides, tried as an alternative source of sulphur, proved rather less effective than ground sulphur when equal quantities of sulphur were applied in each case. The result was probably due to the unsatisfactory state of division of our sample of spent oxides.

Sulphur inoculated with *Thiobacillus thiooxydans* showed no increased efficiency over uninoculated sulphur on Ormskirk soils and appeared less effective than the latter on the Hatfield clay.

The elimination of Wart Disease in the field by sulphur and sulphur compounds is not a simple function of the final soil reaction and it would appear that some sulphur product other than sulphuric acid is the active fungicidal agent.

The sulphur treatment will be put to a large scale critical test in 1925–6; but the results to date seem to show that a feasible method of eradication of Wart Disease from contaminated land has been found.

We wish to express our gratitude to the Piccard Pictet Company for the loan of a 4 H.P. Simar Rotary Tiller and for their assistance on sundry occasions in the working of the machine: to Messrs Chance and Hunt of Birmingham for the donation of much of the sulphur used in our work; to Messrs Carbolimo for a donation of ground limestone; to the National Institute of Agricultural Botany, Cambridge for the use of land at Ormskirk; to His Grace the Marquis of Salisbury for the use of land at Hatfield. Finally we would thank Mr H. B. Bryan and his assistant Miss Whitehead of the Potato Testing Station at Ormskirk; without their expert knowledge, which was freely placed at our disposal, and without their friendly forethought and generous help on innumerable occasions during 1920–4 it would have been impossible for us to carry on our work.

REFERENCES.

(1) Crowther, E. M. (1925). *Journ. Agric. Science*, xv, p. 201.

(2) Eriksson, J. (1914). *Journ. Bd. Agric. London*, xxi, p. 135.

(3) Gimingham, C. T. and Spinks, G. T. (1919). Soil Sterilisation. *Univ. Bristol Agr. and Hort. Res. Sta. Ann. Rep.* p. 37.

(4) Glynne, Mary D. (1925). Infection Experiments with Wart Disease of Potatoes. *Annals of Applied Biology*, xii, p. 34.

(5) Hartmann, R. E. and McCubbin, W. A. (1924). Potato Wart. *Pennsylvania Dept. Agr.* vii, No. 15, *Bull.* 394.

(6) Kunkel, L. O. and Orton, C. R. in Lyman, G. R., Kunkel, L. O. and Orton, C. R. (1920). Experiments in Soil Sterilisation for the Eradication of Potato Wart. *U.S. Dept. Agri. Circ.* cxi, p. 19.

(7) Lipman, J. G., McLean, H. C. and Lint, H. C. (1916). Sulphur oxidation in soils and its effect on the availability of mineral phosphates. *Soil Science*, i. pp. 533–539, ii, pp. 499–538.

(8) Malthouse, G. T. (1914). Wart Disease of Potatoes. *Harper Adams Agr. Coll. Bull.* No. 8.

(9) Martin, W. H. (1921). A Comparison of Inoculated and Uninoculated Sulphur for the Control of Potato Scab. *Soil Science*, xi, pp. 75–84.

(10) Schaffnit, E. and Voss, G. (1916). Versuche von Bekampfung des Kartoffekrebses. *Zeitschr. f. Pflanzenkr.* Bd. 26, p. 183. (1917). *Ibid.* Bd. 27, p. 339. (1919). *Ibid.* Bd. 28, p. 111.

(11) Schaffnit, E. (1920). *Zeitschr. f. Pflanzenkr.* Bd. 30, p. 59.

(12) Snell, J. (1918). Ormskirk Potato Trials. Ann. Rep. 1918 in *Supplement* No. 18 to *Journ. Bd. Agric. London*, March, 1919, p. 68

(*Received February* 1*st*, 1925.)

MAINTAINED GROWTH RATES IN FUNGUS CULTURES OF LONG DURATION[1]

By HOWARD S. FAWCETT.
(*University of California.*)

(With 2 Text-figures.)

In some earlier experiments with certain citrus-disease fungi (Fawcett, 1921), the question arose as to what effect long-continued growth under nearly constantly maintained favourable conditions might have on the rate of advance of the mycelium. The earlier tests for periods of from three to six days, and especially at higher temperatures, had suggested that the time factor has an important influence on the rate of growth.

The results obtained in the tests just mentioned led to an experiment in which two fungi were cultured in the ends of long glass tubes and allowed to advance toward the opposite ends for a period of about four months in one case and six months in the other. The glass tubes were about 5 centimetres in diameter and about 1·5 metres in length. The tubes were sterilised with steam, both ends plugged with cotton, placed horizontally and a sufficient amount of hot culture medium was then run in to produce an agar surface 40 mm. wide extending along the bottom of the tube from end to end. The strip of medium was horizontal, bounded on each side by the supporting walls of the large glass tube. The cotton plug permitted gas exchange with the outside air but prevented contamination. There was a small loss of water by evaporation through the plugs, approximately 2 gm. per week. The tubes were kept in a dark basement room in which the temperature fluctuations were small (Fawcett, 1924). The culture medium was corn meal agar, the same as had been used for the earlier experiments.

The fungi used were *Pythiacystis citrophthora* Sm. and Sm. and *Diplodia natalensis* Evans, which cause rotting of citrus fruits. Their growth-temperature relations had been previously tested for a wide range of temperatures and for shorter periods. After hanging the two large culture tubes in a horizontal position from the ceiling in the dark

[1] Paper No. 120, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

room with the free plane surface of the solidified medium upward, a mycelial disc of *Pythiacystis* 2·5 mm. in diameter was placed in the end of one and a similar disc of *Diplodia* in the end of the other.

The transfer of *Pythiacystis* mycelium was made on Nov. 13, 1917, and the first week of measurement began Nov. 15 when the radial advancement was 5 mm. The transfer of *Diplodia* was made Nov. 27, its first week of measurement starting Nov. 29 when its radial advancement was 4 mm. The growth increments were measured at intervals of one to three days and the maximum and minimum temperatures of the room recorded.

The mycelial disk enlarged as usual forming a circular mat the margin of which reached the lateral walls of the glass tube before the end of the first week, after which its advance was along the surface of the narrow strip of medium toward the opposite end. There was thus, from this time on, an advancing front over and through a uniform medium of equal width and depth. Growth occurred for the most part only along the upper surface of the medium, only a thin upper layer being occupied. Only vegetative hyphae were produced in either case. The *Diplodia* developed some aerial hyphae as it proceeded, but the *Pythiacystis* hyphae were confined almost exclusively to the exposed surface of the medium. The *Pythiacystis* experiment was terminated at the end of 16 weeks because of a contamination at the far end of the tube, but the *Diplodia* experiment was continued for six months and was in perfect condition when it had to be terminated for lack of additional time.

These experiments were carried out in the dark-room of the Laboratory of Plant Physiology of the Johns Hopkins University, at Baltimore, in 1917 and 1918. The writer wishes to acknowledge many helpful suggestions received from Dr Burton E. Livingston of that laboratory.

The growth rate and age of culture.

The results of this experiment expressed in weekly average temperatures and the corresponding weekly increments of growth are shown in Table I.

Table I.

Weekly increments of radial advance of mycelial mat and mean weekly temperatures.

Consecutive weekly periods	Temperature: Daily average minimum ° C.	Temperature: Daily average maximum ° C.	Temperature: Mean ° C.	Weekly increments of advance: *Pythiacystis citrophthora* mm.	Weekly increments of advance: *Diplodia natalensis* mm.
1	18·0	18·6	18·3	24	—
2	15·8	17·2	16·4	25	—
3	15·4	16·5	16·0	25	27
4	13·2	14·5	13·8	21	20
5	13·1	14·4	13·7	19	18
6	14·5	16·3	15·5	27	23
7	12·0	13·5	12·8	19	12
8	14·2	15·3	14·8	26	20
9	14·5	15·6	15·1	29	23
10	13·5	14·6	14·1	25	18
11	12·5	13·8	13·1	18	16
12	13·0	14·3	13·7	21	18
13	14·7	16·3	15·5	27	26
14	13·7	15·0	14·3	25	22
15	15·2	16·5	15·9	29	26
16	16·4	17·5	16·9	33	30
17	17·4	18·4	17·9	—	41
18	16·0	17·4	16·9	—	34
19	16·0	18·0	17·0	—	40
20	13·8	15·0	14·4	—	24
21	14·8	15·4	15·1	—	24
22	14·2	14·6	14·4	—	18
23	14·5	15·7	15·1	—	24
24	17·3	18·7	18·0	—	40
25	18·4	19·2	18·8	—	42
26	19·7	20·5	20·1	—	60
27	20·5	21·7	21·1	—	69

There is to be noted (Table I) a small fluctuation of average temperature from week to week, but the changes were always comparatively slow. The usual daily fluctuation was only about 0·5° C. The difference between the highest and lowest weekly mean was only 5·2° C. for the 16 weeks of the shorter experiment and 8·3° C. for the 25-week period of the other.

It is evident from Fig. 1 that after an initial adjustment there was a fairly consistent growth, fluctuating with the temperature and apparently independent of the lapse of time. There is no suggestion of anything corresponding to a "grand period of growth" nor any indication

of "staling" nor of influences causing the mycelial growth to follow the course of an autocatalytic reaction with the lapse of time (Reed, 1920, 1924; Robertson, 1923). The growth rate for any period is nearly the same as that for any other weekly period having the same temperature mean, irrespective of the time factor. For example, an average temperature of 15·5° C. occurred on the 6th and again on the 13th week and was accompanied by a growth of 27 mm. in each case. There was a temperature of 13·8, 13·7 and 13·7° C. on the 4th, 5th and 12th weeks with a growth of 21, 19 and 21 mm. respectively. With *Diplodia* there was a temperature of 15·1° C. recorded for its 7th, 19th and 21st weeks, with increments of 23, 24 and 24 mm. respectively. The correlation

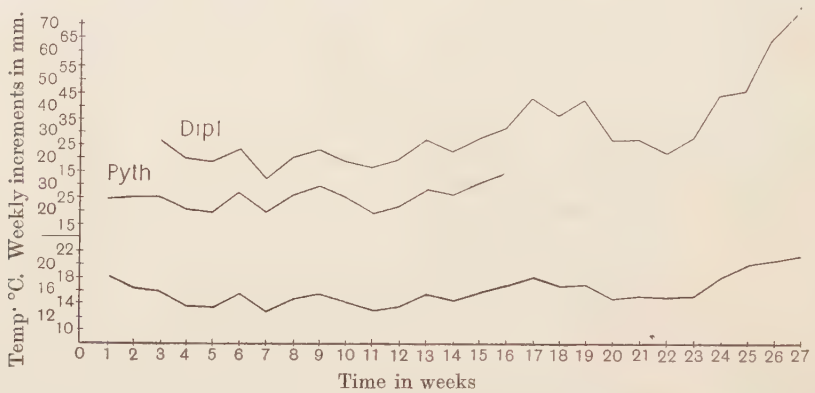


Fig. 1. Graphs of weekly growth increments for *Pythiacystis* and *Diplodia* mycelia kept constantly growing on long strips of corn-meal agar, also the corresponding weekly means of temperature.

coefficient of growth with temperature is ·904 ± ·03 for *Diplodia* and ·699 ± ·09 for *Pythiacystis*.

It is thus apparent that these fungi show no tendency toward senility as long as the environmental conditions encountered by the advancing hyphae at the outer margins are within such limits as were provided by these experiments. There is ample reason for supposing that these tests might have been continued for many years if the agar tubes had been long enough and if temperature fluctuations had been about what are shown for the 25-week period under consideration. Of course some form of senility might appear if the period were long enough. It seems probable that vegetative growth may generally be intrinsically immortal in this way. With maintained temperature around 15–20° it seems probable that these fungus mats would have advanced at a uniform rate as far as the agar medium extended.

These conclusions may seem, at first thought, to constitute an exception to the general rule for plant and animal growth, which is characterised by the phenomena of the grand period and grand march, with initial slow growth, followed first by an acceleration and then by a retardation, with a final cessation of the process. If individual hyphal cells of these fungi were considered, however, it seems probable that each cell would exhibit the usual behaviour of an autocatalytic reaction. The careful measurements of growth rates of individual fungus hyphae of *Botrytis cinerea* by J. Henderson Smith (1924) showed that when the period was extended under the usual conditions of branching, the growth rate of single hyphae was at first slow, then steadily increased to a maximum and later became slower and slower. The measurements however, of the "whole hyphal system, *i.e.* the parent hypha with all its branches and sub-branches," showed the growth rate for the whole as a unit to be constant for hours at a time. The lack of conformity to the autocatalytic rule in the growth of the mycelium as a whole is probably due to an unusual condition where the environment, externally and internally, with the exception of a slight fluctuation of temperature, was effectively the same throughout the experiment. Since elongation in such filaments takes place only at the hyphal tip (Smith, J. H., 1923, 1924), the tips found themselves always in the same environment, with the exception of environmental fluctuations based on temperature changes.

In higher plants under usual conditions the relation between protoplasm and its surroundings is altered with time, due to the accumulations of growth-modifying or growth-retarding substances, etc., in the living mass. This aspect of the subject is clearly discussed by Reed (1924, p. 343). In such cases the usual autocatalytic curve may be thought of as made up of an infinite number of small ones. For example, in the elongation of a sunflower stem or of a citrus or pear shoot which have been shown by Reed (1919, 1920) to follow an autocatalytic curve we may think of any single row of individual cells parallel to elongation as having followed in their own growth an autocatalytic curve. Each individual cell, then, has a curve which is a part of the larger curve of the same type representing the elongation of the shoot as a whole.

Unlike a complex plant or animal in its growth the margin of these hyphal mats did not materially change its environment by the very fact of growth. It left behind it a mat of tissue which doubtless was different from that near the margin. If a single line of the same width as one hypha were considered moving parallel with the tube, and the details of the entire elongation were represented by a graph, it would be made

up of a chain or series of very small autocatalytic curves each beginning where the other left off. Where the growth rate of the whole is constant a smoothed graph through this chain would, of course, be a straight line.

When organisms of this kind are grown in liquid medium in flasks, where the mycelium is unable to advance beyond the accumulated products of growth and where the concentration of nutrient substances alters with growth because of previous growth reactions, then the growth rate for the mycelium as a whole as well as for each individual cell must, of course, behave as an autocatalytic reaction. It has been shown

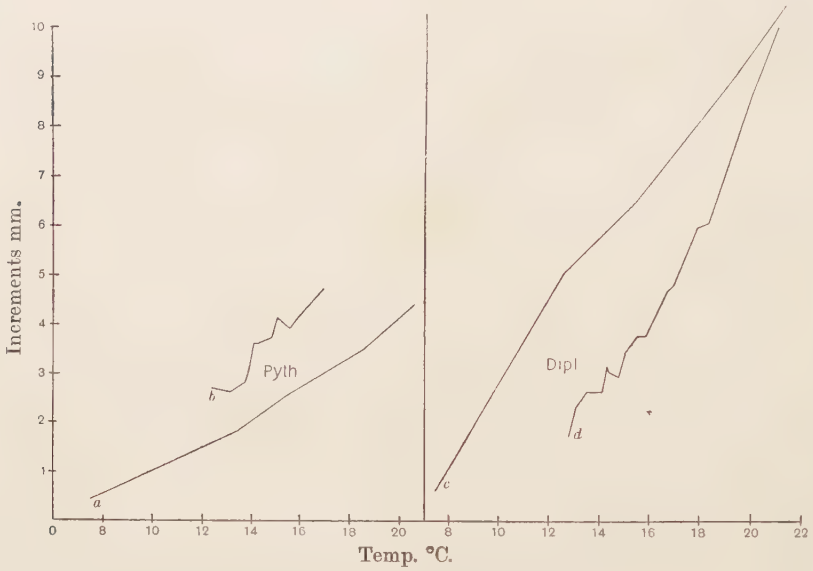


Fig. 2. Graphs comparing the relation of elongation of mycelium to temperature in short-time and long-time experiments.

Pythiacystis citrophthora. (*a*) Average daily radial increments for first five days at different maintained temperatures (Fawcett, *Univ. of Calif. Publ.* vol. IV, p. 201, Table I). (*b*) Average daily linear increments as related to temperature during the four-month experiment, derived from Table II of this paper.

Diplodia natalensis. (*c*) Average daily radial increments for first three days at different maintained temperatures. (Citation above, p. 204, Table IV.) (*d*) Average daily linear increments as related to temperature in the six-month experiment, derived from Table II of this paper.

that the rate of multiplication of bacteria follows the general course of an autocatalytic reaction (Miyake, 1916). But there is no reason to suppose that this would be true if the medium of a bacterial culture were continually renewed, as was automatically true for the present fungus cultures. By the very nature of bacterial cultures, the medium

is not thus renewed, unless the bacterial cells move forward into new media as rapidly as they produce significant alteration in their neighbourhood. Apparently they do not generally do this, but it may well be that a medium and maintained temperature might be arranged so that a bacterial colony would behave as did these fungus mats. The spatial relations of growth are also different in the liquid medium from that of the fungus in the tube.

GROWTH RATE AS INFLUENCED BY TEMPERATURE WHEN THE TIME FACTOR IS ELIMINATED.

When the average weekly temperatures, irrespective of when these occur in the consecutive series, are placed as in Table II—in the order of their magnitudes omitting the first two weeks—the range is from 12·8 to 16·9° C. for *Pythiacystis* and from 12·8 to 21·1° C. for *Diplodia*. In deriving the figures for comparison the average daily increase is calculated from the weekly increments and this compared with the radial increase (one-half of the diameter increase) of mycelial mats of Tables I and IV of the previous publication (Fawcett, 1921). For *Pythiacystis* the daily averages for the first five days are used, but for *Diplodia* only those for the first three days are available.

Table II.

Weekly growth increments arranged in the order of magnitude of the weekly means of temperature.

Weekly periods	Mean temperature ° C.	Weekly growth increments	
		Pythiacystis mm.	*Diplodia* mm.
7th	12·8	19	12
11th	13·1	18	16
5th and 12th	13·7	20	18
4th	13·8	21	20
10th	14·1	25	18
14th	14·3	25	22
20th and 22nd	14·4	—	21
8th	14·8	26	20
9th, 21st and 23rd	15·1	29	24
13th	15·5	27	26
15th	15·9	29	26
16th	16·9	33	30
19th	17	—	40
17th	17·9	—	41
24th	18	—	40
25th	18·8	—	42
26th	20·1	—	60
27th	21·1	—	69

The weekly increments corresponding to these temperatures, plotted against the average temperatures as abscissae (Fig. 2), follow a curve similar to that given by the earlier experiments (Fawcett, 1921). The exact position of the graph, however, is different. In *Pythiacystis* the graph derived from the average weekly increments is higher and in *Diplodia* it is lower than in the experiment for the shorter growth periods.

There is naturally a good deal of "experimental error" to be considered in an experiment like this. One phase of this error lies in the employment of weekly mean values of temperature. It is probably seldom true that the physiological influence of a changing temperature is quantitatively equivalent to the influence of a maintained temperature whose magnitude is that of the mean between the minimum and maximum temperatures. The method by which the small temperature fluctuations of these experimental tests are handled might be greatly improved by greater precision in temperature control.

The outstanding point is, as already emphasized, that these fungus cultures generally showed fluctuations in growth rate that corresponded to temperature changes, in about the way that would be expected from the earlier-published data, and that these cultures failed to show any significant changes in growth rate that cannot be definitely related to corresponding temperature changes, and not to the influence of the time factor.

REFERENCES.

Fawcett, H. S. (1921). Temperature relations of growth in certain parasitic fungi. *Univ. of Calif. Publ. Agri. Sciences*, iv, 183–232.

—— (1924). Influence of time and temperature on rate of growth of certain fungi. *Abst. in Phytopath.* xiv, 119–120.

Miyake, K. (1916). The nature of ammonification and nitrification. *Soil Science*, ii, 481–492.

Reed, H. S. (1920). The nature of the growth rate. *Journ. Gen. Physiol.* ii, 546–561.

—— (1924). The nature of growth. *Am. Naturalist*, xlviii. 337–349.

—— and Holland, R. H. (1919). The growth rate of an annual plant, *Helianthus*. *Proc. of Nat. Acad. Sciences*, v, 135–144.

Robertson, T. B. (1923). The chemical bases of growth and senescence. 389 pp. *Monograph on Experimental Biology*. J. B. Lippincott.

Smith, J. Henderson (1923). On the apical growth of fungal hyphae. *Annals of Botany*, xxxvii, 341–343.

—— (1924). On the early growth rate of the individual fungus hypha. *New Phytologist*, xxiii, 65–79.

(*Received December 12th*, 1924.)

REVERSION DISEASE OF BLACK CURRANTS: MEANS OF INFECTION

By A. H. LEES, M.A.

(*Agricultural and Horticultural Research Station, Long Ashton, Bristol.*)

(With Plates XII–XIV and 1 Text-figure.)

The following paper deals mainly with work that has been in progress for some years, but which, from its nature, did not admit of a speedy culmination. Statistical observations have been continued this year with the assistance of Mr C. E. T. Mann, Plant Physiologist at Long Ashton, who is responsible for the presentation of the evidence relating to them. The investigation of the disease has long called for the cooperation of a competent physiologist as there are many promising lines which can only be fully explored by modern methods.

In previous papers dealing with Reversion in Black currants attention has been directed to such points as the characters of the disease (1), means of identification (2), possible connection with the Black currant mite, *E. ribis* (3), and control. At the same time however various experiments were started with a view to elucidate its means of entry into the plant. The most obvious line was to investigate by direct experiment the influence of an infection of mite on healthy plants. Cases of disease however were comparatively frequent where no possible connection could be traced between mite and the infection of reversion. Thus it was by no means infrequent to find new cases of reversion arising in which either no mite could be found or mite was present in such small quantity as to suggest that it was not the true cause.

Grafting Experiments.

Accordingly in order to test whether the disease could be propagated trials of grafting were started in the year 1920, and were continued in 1921. These first two years, for various reasons, gave only failures. It was not then easy nor has it proved easy subsequently to obtain grafts on the black currants. The graft can be made but a successful union is often not obtained. On the only successful grafts of 1920 a tree was felled so that even where there was a chance of obtaining a positive

result this chance was ruled out by accident. This sort of experience was repeated in a similar manner for grafts on plantation bushes where in most cases before results could be obtained the graft was knocked off or out by cultivating tools or the whole situation was complicated by the arrival of big bud.

Owing to these various reasons it was decided to make grafts very largely on pot trees so that the plants could be preserved free from accident and if possible from mite infection, until either positive or negative results could be recorded.

Table I sets out the results obtained from grafting reverted scions on healthy stocks.

Table I.

Effect of grafting diseased scions on healthy stocks.

No.	When grafted	With what	Same year plant	2nd year		B.B. Present	3rd year	
				Flowers	Shoots		Flowers	Shoots
1	Spring 1922	Revert Boskoop 2. 1. 13	N	N	N	No	N	N except shoot near base
2	„	Do. 2. 3. 21	„	„	„	„	R	R
3	„	„	„	„	„	Yes on graft	„	„
4	„	Do. 2. 1. 13	„	„	„	No	Part R „ N	R except shoot near base
33	„	Revert Seabrook 6. 1. 13	„	„	„	„	None	One shoot Rev. 6
36	„	„	„	„	„	„	„	Some leaves Rev. 4
34	„	„	„	„	„	—	—	—
35	„	„	„	„	„	—	—	—
14	Spring 1923	Revert Boskoop 2. 5. 6	Some temporary reversion	„	R	No	—	—
15	„	„	„	„	R and N	„	—	—
16	„	„	„	„	„	„	—	—
17	„	„	„	„	N	„	—	—
18	„	„	„	„	R and N	„	—	—
19	„	„	„	„	R	„	—	—
20	„	„	„	„	R and N	„	—	—
21	„	„	„	„	„	„	—	—

In every case the stock plants were kept under observation for one or two years previously in order to rule out, as far as possible, all chance infection.

The 1922 grafts were made with grafts from three different sources, two being Boskoop Giant and one Seabrook's Black. In each case the

stock used was the same variety as the scion. Of the eight 1922 grafts six ultimately produced reversion on the stock by 1924, while two were still free in 1923 but were lost during the operation of repotting. The original bush Seabrook 6. 1. 13 was only slightly affected at the time that the grafts were cut, and this weakness reappears in the stock to which the disease has been communicated. In the two successful cases, though reversion showed on a shoot in each case, the intensity, judged by the leaf-vein method, was slight (veins 6 and 4).

Disease on Boskoop 2. 1. 13 and 2. 3. 21 was apparently of equal intensity when the selection of grafts was made, but their subsequent history suggests that the disease 2. 3. 21 was stronger. In this case by 1924 the shoots and flowers from the stock were fully revert with the exception of one basal shoot in one of the two plants.

The 2. 1. 13 grafted stocks in 1924 still showed a fair proportion of normal shoots and flowers, though the disease had quite evidently appeared on some. In only one of these eight 1922 grafts did Big Bud appear and this was on a graft and not on the stock.

The 1923 grafts were all Boskoop on Boskoop and the bush selected for grafts was of a pronounced revert type. In the summer of 1923 all the eight grafted stocks showed well-marked temporary reversion due to the cutting back that was done after grafting. There is nothing to suggest that the disease made a quick infection from graft to stock during the year of grafting, and the results for 1924 show that even then the disease was not spread throughout the stock. In no case were any of the flowers on the stock shoots revert. The leaves however showed the beginning of the spread of the disease. In number 17 no infection had been made. In 15, 16, 18, 20 and 21 some shoots were revert and some normal. In 14 and 19 the shoots were all revert. The intermediate cases, where both normal and revert shoots were produced from the stock, were particularly interesting, as in them the course of the disease could be distinctly traced. Fig. 1 illustrates the result obtained for number 15. The shoot behind the graft, which did not "take," is clearly reverted, while the shoot issuing a little farther away was still healthy when the photograph was taken. This slow progression of the disease is also shown in Figs. 2 and 3, but especially in 2, where the shoot immediately behind the graft was most revert, the next one less so, and the one farther away quite free. In all these 1923 cases the graft failed to "take," but this fact did not prevent the propagation of the disease though it may have lowered its rate of spread.

These sixteen grafted plants would appear therefore to suggest the following conclusions:

(1) That reversion can be propagated by contact.

(2) That it is therefore an organic and not a functional disease.

(3) That since no organism has been found after many attempts the disease probably belongs to the class of virus diseases.

(4) That it is propagated slowly from the point of infection downwards, thereby infecting shoots arising lower than the point of infection.

(5) That the rate of propagation and the intensity of the attack depends directly on the intensity of the original infection.

(6) That its propagation *can* be quite independent of the presence of Black Currant Mite.

Statistical Observations.

The season 1924 has been remarkable for the large increase in the number of fresh cases of reversion. Statistical investigation of the disease, continued on a plantation of 547 bushes comprised of Hatton's four main groups, showed a percentage increase of approximately 7 per cent. in Baldwin (Strain I), 9 per cent. in Edina, 11 per cent. in Seabrook's Black, 13 per cent. in Boskoop Giant and 23 per cent. in Baldwin (Strain II). The results of the investigation are summarised in Table II and the percentages quoted above are calculated on the number of healthy bushes remaining in the various groups at the time of the last marking, namely the foliage marking in 1923.

As stated in a previous paper(5), up to 1923 the number of new cases of the disease was steadily decreasing and only four fresh cases, less than 1 per cent., were observed in that year.

Table II.

Variety	Number of bushes 1920	Reverts				Increase in number of reverts			Remaining healthy	Percentage increase
		1921	1922	1923	1924	1922	1923	1924	1923	1924
Edina	115	28	26	25	33	3	−1	8	90	8·9
Boskoop Giant	123	5	6	7	22	1	1	15	116	13·0
Seabrook's Black	134	6	8	10	24	2	2	14	124	11·2
Baldwin I	57	0	0	1	5	0	1	4	56	7·1
Baldwin II	118	4	7	7	32	3	0	25	111	22·5
Totals	547	38	47	50	116	9	3	66	497	13·27

The bushes were carefully marked in April and early May, according to variety, for the presence of the typical "revert inflorescence" and in June for signs of the disease exhibited by the terminal foliage (2). A total of 77 fresh infections resulted from the two markings, the total number of affected bushes being 66. The difference between these two figures may be accounted for by the fact that it is quite possible to find two twigs or branches on the same bush remotely connected through the stool and showing the presence of the disease in differing intensity and at different periods of the season, a condition which indicates separate infections.

Considering the flower markings made in April and May, of a total of 25 twigs and branches bearing revert inflorescences 19 produced revert terminal foliage later in the season. Infection varied from a single truss in slight cases to all the trusses on a fruiting twig. The extent of the variation is shown in Table III.

Table III.

Summary of new infections.

	Presence of reversion established on :—									
	Flower mark. only	Flower and foliage marking					Foliage marking only			
		Twig				Branch	Twig	Branch	Stool shoots	
Variety	One rev. truss N. foliage	Isolated R. truss. Terminal fol. R.	Upper trusses R. Terminal fol. R.	Lower trusses R. Terminal fol. R.	All trusses R. Term. fol. R.	Whole branch R. infs. and fol.	Terminal foliage R.	Terminal foliage of main and laterals R.	and laterals arising near pruning cut	Totals
Edina	1	0	2	1	2	0	0	2	0	8
Boskoop Giant	1	0	2	2	1	2	6	4	1	19
Seabrook's Black	0	0	0	0	1	1	10	1	3	16
Baldwin I	1	0	0	2	0	0	0	1	0	4
Baldwin II	3	1	0	1	0	1	11	6	7	30
Totals	6	1	4	6	4	4	27	14	11	77

Where only a single truss appeared to be infected, reversion in six cases did not appear later in the foliage. It will also be observed that four of the six cases occur in the Baldwin Group where recognition of the disease in the inflorescence is much less easy than in a variety such as Boskoop or Seabrook.

The figures obtained on the foliage marking in June illustrate again the possibility of "dosage" or varying severity of infection. Of the total of 52 fresh cases of disease 27 are single twig infections where the terminal foliage in June shows the reduced venation and coarsened margin characteristic of the disease. More severe cases were noted where

the terminal foliage on all the current year's growth of a branch was reverted. In addition to the above cases, instances of possible infection through a bad pruning wound were recorded. One case, in Seabrook's Black, showed very clearly the possibility of such an infection taking place. A strong stool shoot of the previous year which had been badly snagged had later in the season produced three strong laterals. Of these, two had arisen near the cut and were strongly revert while the third, which had its origin at a considerably lower level, was quite normal (Fig. 4). This case seems to form a striking parallel with the case of the grafted plant (Figs. 1 and 2) in which the shoot arising nearest the point of infection by the revert scion has reverted before the shoot of more distant origin.

The subsequent behaviour of bushes marked for revert inflorescences is worthy of special note. In all cases where one or more trusses had been marked for reversion in April the June observations showed either a complete absence of fruit or the presence of a very few small unripened berries on the infected trusses. On the other hand, the June marking showed numerous cases of fruiting twigs and branches carrying a full crop but showing strongly revert terminal foliage on the current year's growth (Fig. 5). It has been suggested that nursery beds may be started free from reversion disease by selecting cutting material only from fruiting bushes. The foregoing observations show that the chance of including revert material is not eliminated by this method and in actual practice usually results in the appearance of about 4 per cent. reverts in the nursery beds.

The relative distribution of previously reverted and newly infected bushes in the plantation is represented in text-fig. 1. An examination of the chart reveals no definite relation between old reverts and new infections; the distribution of the latter seems quite haphazard.

In the chart (text-fig. 1) the pruning treatments applied are shown. In addition the first eight rows, including the three pruning methods employed, received a lime sulphur spray of 1 in 12 as soon as the leaves were as large as a sixpence. The next four rows received four sprayings of lime sulphur at 1 in 40 at intervals of ten days. From the thirteenth row the pruning treatments are repeated, but the bushes were not sprayed.

The results summarised in Table IV show firstly that lightly pruned bushes were more liable to reversion than were hard pruned bushes, secondly, that spraying with lime sulphur had a beneficial effect, especially if the spray was applied at summer strength at four intervals of 10 days, but little benefit accrued from spraying unless the bushes received the usual pruning.

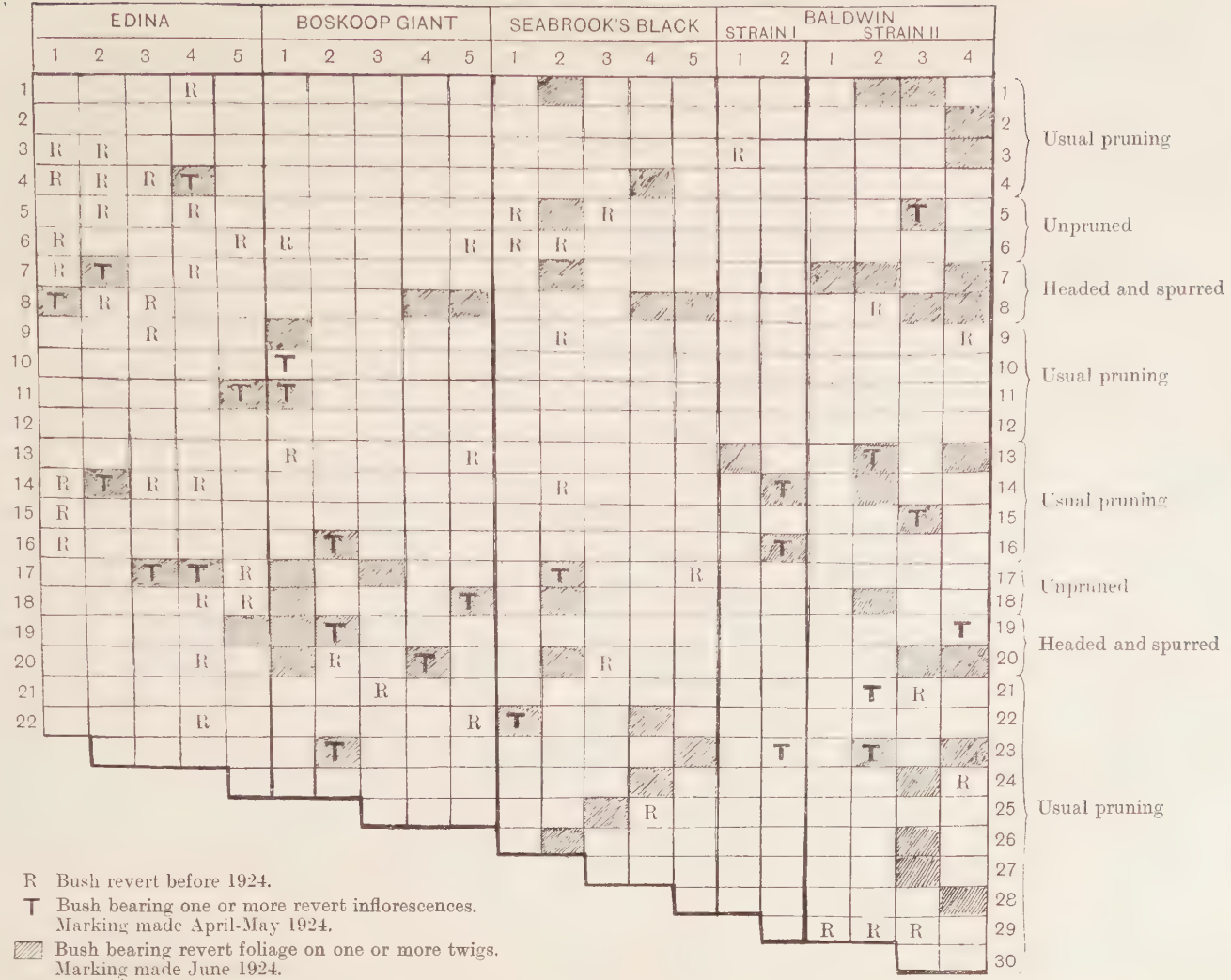


Fig. 1. Chart showing general lay out, treatments and results in the trial plantation.

Table IV.

Spraying Treatment	Percentage new infections			
	Rows	Hard pruned	Rows	Light pruned
Lime-sulphur 1 in 12	1–4	9·52	5–8	21·06
„ 1 in 40	9–12	3·22	—	—
Unsprayed	13–16	13·11	17–20	22·95
„	21–end	13·46	—	—

During the season 1923 the plantation was badly attacked by aphis. From observations made at the time, aphis damage was much less severe on the bushes receiving four sprayings of lime sulphur than on any others. These observations would appear to suggest the possibility of aphis acting as a carrier of the disease, and would explain to some extent the haphazard distribution of new infections appearing in 1924. This suggestion is supported also by the evidence from grafting experiments where it is shown that contact with revert material is sufficient for the propagation of the disease in a healthy plant. In the absence of direct evidence the effect of hard pruning in reducing the number of infections can only be surmised, but it may be possible by pruning to remove many mildly attacked shoots before the disease has reached the region where the pruning cut is made.

From the foregoing observations the following main conclusions may be drawn with regard to the new infections of reversion disease for 1924.

(1) Infection with the disease may vary in severity between wide limits.

(2) There is no evidence of occurrence of the disease solely on the ground of proximity of healthy and infected plants.

(3) The possibility of infection by contact with diseased material during the process of pruning is suggested.

(4) There is some evidence in support of the possibility of a pest carrier.

(5) Hard pruning with lime sulphur spraying had a considerable control influence.

To test the evidence obtained from observations in the plantation work has been commenced on the following lines:

I. Injection of the expressed fluid from a revert plant into various parts of a normal plant.

II. Ringing and subsequent introduction into the ringed healthy shoot of the extract from revert shoots.

III. Propagation of green cuttings from a healthy bush previously infected with the fluid obtained from corresponding regions of a revert bush.

IV. Attempted pruning cut infections in the field.

V. Hard wood healthy cuttings treated with extracts obtained from diseased plants.

Possible carriers of the disease.

It is clear therefore that amongst the cases described in the above section there are a large proportion which cannot be explained as infection by contact. Nevertheless it is obvious that the disease has obtained entry by some means, since those bushes prior to 1924 were healthy.

Undoubtedly the easiest hypothesis to accept is that the disease can be carried by some sucking arthropod from diseased to healthy bush in the way that is already familiar in several virus diseases (Potato Aphis, *Eutettix tenella* and Curly Top of Sugar Beet, etc.).

At first sight, in view of the frequent close connection of mite with reversion, this arthropod might be expected to convey the infection. This connection is however explicable in two ways. Mite may cause or carry reversion or reverted bushes may be more susceptible to mite. That the latter contention is true has been shown by statistical studies already published (4). But this does not preclude the truth of the first contention. The evidence for this is at present conflicting and is derived from two sources, correlations in the field and direct infections. The correlations were derived from two varieties, Edina and Baldwin, now under observation for four years. In the group of Edinas under inspection there are 115 bushes and of these 40 have been recorded as big budded 1 year out of 4, 19 big budded 2 years out of 4, 5 big budded 3 years out of 4, and 1 big budded all four years and yet all have remained free from reversion for all four years referred to. It is therefore clearly possible for mite to be present for any period from one to four years without the bush becoming revert. A lack of correlation is also shown in the Baldwin Group (Strain II) between mite infections recorded in 1923 and new cases of reversion appearing in 1924. Out of 111 bushes remaining healthy in 1923, as regards reversion, 25 showed the presence of the disease in 1924. On these figures the probability of a bush of this variety reverting in the past season was 25/111 or 22·5 per cent. Of the total of 111 bushes not revert in 1923, 28 were found to be mite infected

in December of that year and of these 7 were revert in 1924. The probability of a mite attacked bush becoming revert is 7/28 or 25 per cent. These two figures for probability of infection indicate a very slightly higher probability in the case of mite infected bushes, but the difference between the two figures, namely 2·5 per cent., is much too small to be significant and is well within the limits of experimental error.

The evidence for mite producing or carrying reversion is derived from direct infection experiments. In Table V are shown the results obtained by infecting healthy pot plants with mite. There are six cases where an attempted infection produced reversion in the plant and three cases where Big Bud was produced but no reversion, and six cases where no Big Bud and no reversion were produced. Thus where no Big Bud followed no reversion was obtained and from eight successful Big Bud infections only five successful reversion infections followed; where reversion was produced in two cases it appeared in the same year, and in three cases in the following year. The numbers in this experiment are admittedly very small and certainly too much reliance must not be placed on them. They do appear to show however that a successful infection of Big Bud is followed by reversion though not necessarily in the same year in which the Big Bud appears and certainly not usually in the year of mite infection. The contention is supported by a field trial where on 15 healthy Edinas 10 infections of mite were made. In the first year one of the bushes showed typical mite revert foliage (tomato leaf type, Pl. XIV, fig. 6). The result has already been obtained by Massee at East Malling and in this respect this infection confirms his claim. The Long Ashton field infection trial will need to wait for a year or two more before conclusions can be drawn.

Summing up therefore the evidence, it would appear that mite infection may produce reversion (either directly or by being a carrier) though there are many cases to be found where no reversion has followed the appearance of Big Bud. It is obvious that further work is necessary on these points, especially in respect of testing possible reversion carrying power of mite.

Whether subsequent experiments prove that mite does or does not carry reversion infection does not affect the possibility that arthropods other than mite may be carriers. Black currants are attacked commonly by two species of aphis and also fairly frequently by capsids.

The case for aphis is suggestive. It is at least remarkable as pointed out in that part of the paper dealing with statistical results that a bad aphis year was followed by a sudden marked increase in the number of

Table V.

Relation of mite infection to the production of reversion.

No.	Big bud appeared in			Reversion appeared in			Reversion did not appear
	1st year	2nd year	3rd year	1st year	2nd year	3rd year	
5	.	×	.	.	.	×	.
8	×	.	.	.	×	.	.
10	.	.	×	.	.	×	.
11	×	.	.	.	×	.	.
29	×	.	.	×	.	.	.
30	.	.	.	×	.	.	.
37	×	.	.	.	.	.	.
38	.	×	.	.	.	.	×
43	.	.	.	.	.	.	×
6 cases did not appear			.	.	.	.	.

new reverts (1 per cent. new cases in 1923, 13 per cent. in 1924 in round figures). I am indebted to Mr W. P. Seabrook for a similar observation bearing out this contention. In 1918 in his experience a bad attack of aphis was followed by a very marked increase of reversion. In a bad year the pest is never completely controlled and consequently winged parthogenetic females are produced in numbers. In a mild year by the time these are due to appear spraying and natural enemies have usually greatly reduced the number of parent aphids. Consequently but few winged forms appear. It is of course only winged forms that can convey disease from revert to healthy bush since the wingless ones do not move from bush to bush.

There is however as yet no direct proof, but it will be the aim of future experiments to test whether mite, capsids or aphis be carriers of the disease.

Summary.

(1) This paper is a continuation of previous work on Reversion Disease of Black Currants, and deals more especially with the possible means of infection.

(2) Evidence is put forward showing that the disease can be propagated by contact of diseased material with healthy, either by grafting or by pruning with a contaminated pruning tool.

(3) The disease, after it has attained entry, is propagated slowly downwards.

(4) The rate at which it travels appears to depend on the intensity of the original infection, and there is some evidence to show that the intensity of the resulting disease is also similarly dependent.

(5) Propagation can be independent of Black currant mite, *Eriophyes ribis*.

(7) There is no evidence of occurrence of the disease solely on the ground of proximity of healthy and infected plants.

(8) There is some evidence in support of the possibility of a pest carrier.

EXPLANATION OF PLATES XII—XIV

Fig. 1. Effect of grafting revert graft on healthy black currant plant. The graft has died, but the shoot nearest graft has become revert, the other shoot being still healthy at the time of photographing.

Fig. 2. Ditto. Shoot nearest graft strongly revert, next shoot moderately revert, farthest shoot still healthy.

Fig. 3. As Fig. 1, but not quite so marked.

Fig. 4. Effect of pruning with contaminated knife. The two shoots behind the snag strongly revert, the shoot issuing lower down still healthy. This is a plantation bush and the effect was obtained accidentally.

Fig. 5. Shoot bearing heavy crop with revert foliage, showing that the infection took place too late to infect the flower buds.

Fig. 6. Effect of a heavy artificial infection of mite. The infected shoot has produced revert leaves of the tomato type.

REFERENCES.

(1) Lees, A. H. (1918). "Reversion" and Resistance to "Big Bud" in Black Currants. *Ann. App. Biol.* v, No. 1, July.

(2) —— (1922). Leaf Character in Reverted Black Currants. *Ann. App. Biol.* ix, No. 1, April.

(3) —— (1921). The Association of Black Currant Mite (*Eriophyis ribis*) with Reversion Disease. *Ann Rep. Ag. and Hort. Research Sta. Long Ashton, Bristol.*

(4) —— (1922). Statistical Studies on the Propagation of Big Bud and Reversion Disease of Black Currants. *Ann. Rept. Ag. and Hort. Research Sta. Long Ashton, Bristol.*

(5) —— (1923). *Ann. Rep. Agric. and Hort. Res. Sta. Long Ashton.*

(6) Ridler, Winifred F. F. (1924). Investigation of the Structure of Reverted Black Currants. *Ann. App. Biol.* xi, No. 2, July.

(*Received December 3rd*, 1924.)

Fig. 2.

Fig. 1.

LEES.—Reversion Disease of Black Currant: Means of Infection. (pp. 199—210.)

Fig. 4.

Fig. 3.

LEES.—Reversion Disease of Black Currant: Means of Infection. (pp. 199—210.)

Fig. 6.

Fig. 5.

LEES.—Reversion Disease of Black Currant: Means of Infection. (pp. 199—210.)

STUDIES ON THE SEX-RATIO AND RELATED PHENOMENA

6. THE EFFECT OF POLYGYNY.

By A. S. PARKES, M.A., Ph.D.
(*Beit Memorial Research Fellow.*)
(*From the Department of Physiology, University College, London.*)

CONTENTS.

Introductory.

The importance to agriculturists of the proportions in which the sexes are produced makes it useful to collect data relative to all the factors which appear to influence the sex-ratio at birth, and the experiments described in the following paper were an endeavour to determine on mice the results of polygyny on the proportions of the sexes. While results obtained on mice do not of necessity apply to farm animals, the similarity of many phenomena of reproduction among different mammals makes it likely that conclusions drawn from experiments on animals which can be easily and accurately manipulated may throw light on the larger problems of animal breeding.

In mammals the male is usually monogynous, but this is no doubt due largely to other circumstances than physiological limitation. As far as the relative reproductive capacities are concerned there would be every reason for polygyny in mammals. In some cases mammals have become polygynous, including certain races of man and domesticated animals under man's guidance, and it is in these cases that statistical data relative to the proportions of the sexes resulting from polygynous matings have been accumulated. This fact divides the available data into two clear classes, firstly, comparison of the sex-ratio found in polygynous races of man with that found in monogynous races, and secondly,

comparison of the sex-ratio among the offspring of the males of domestic animals grouped according to the number of females allowed to them in a season.

In the human subject the practice of polygyny usually occurs in conjunction with a female excess in the population, and this connection has probably given rise to some confusion as to cause and effect.

It was at one time supposed that polygyny resulted in a preponderance of females, an assertion made by Montesquieu(6) as early as 1753. In more recent times Burton has quoted to the effect that there is a big excess of females among the Mormons owing to polygyny. This statement, however, begs the vexed question of the extent of polygyny among the Mormons, and in any case Newcomb(7) found a very normal sex-ratio in the Mormon Colony.

In normally polygynous races investigation has revealed nothing extraordinary in the proportions of the sexes at birth. Sanderson(11) found no great peculiarity in the births among the polygynous Kaffirs of Natal, and came to the conclusion that disturbance of the sex-ratio due to polygyny was negligible. Campbell(1) considered the question in the harems of Siam and collected rather interesting data. In 17 harems there were 191 wives with children; or 11·2 mothers per man. The children from these women numbered 440, of which 229 were males and 211 were females. This gives a sex-ratio of 108·5, which can hardly be considered as abnormal, though, unfortunately, the general sex-ratio of the people concerned was not given.

Thomas(12), however, gives statistics which show that in his data, at any rate, the proportion of males is correlated with the number of wives. His figures, relating to the Ibo of the Awka, are as follows:

Table I.

Polygyny and sex-ratio (Thomas).

No. of wives	Percentage of males
1	49
2	51
3	52
4	55
5 +	57

The evidence relative to the human subject is thus indefinite, but in view of the very mild form of polygyny which obtains in man this is not surprising, and, *a priori*, it would be expected that the more pronounced polygyny found in the breeding of farm animals would show

more definite results. Düsing(2,3) approached the question in horses and took the number of times a stallion went to stud in a season as his criterion of polygyny. His summary table is of great interest and may be reproduced here:

Table II.

Amount of stud work and sex-ratio in horses (Düsing).

No. of times to stud	Foals produced		Sex-ratio
	Males	Females	
60 +	71407	70569	101·19
55–59	75493	74912	100·77
50–54	69972	71461	97·92
45–49	69774	72073	96·81
40–44	66573	69045	96·42
35–39	44911	46493	96·60
20–34	29023	29934	96·94
Total	427153	434487	98·31

As the numbers of births are large the results should be correspondingly free from error of chance, and these results appear to indicate that increased demand on the stallions raised the sex-ratio of the offspring. Some consideration is, however, necessary. Even the least degree of polygyny considered in the above table is very considerable when viewed from a monogynous standpoint, and is very much in excess of what can obtain naturally in the majority of mammals, whilst the highest groups in the above table are much more so. In spite of this, however, none of the ratios found depart markedly from that approximate equality between the sexes at birth which seems to characterise mammalian reproduction.

Although I know of no data for bulls, rams, or boars corresponding to the above table, it may be safely said that 20–50 females is the usual allowance for these males, even higher numbers of females being allowed to many rams. As in the case of the horse, however, nothing striking is found in the sex-ratio of the young of such animals. Among both calves and pig litters a slight excess of males seems to obtain, but the excess is so small that the extreme polygyny practised can have little effect.

Polygyny in the Mouse.

The foregoing review of existing data contains no record of experimental work, and the experiments described below were undertaken in the hope of removing this deficiency.

The ordinary matings in my mouse colony were made on a monogynous basis, and the sex-ratio of offspring bred under such condition is near equality. During the last three years 1701 mice have been bred monogynously in the colony, and of these 905 were males and 796 females, giving a percentage of males $53{\cdot}2 \pm {\cdot}81$[1].

For the purpose of investigating the effect of polygyny on the ratio, 8 to 12 females were mated up with one male in a suitably large cage and the sex of the offspring obtained immediately after birth by dissection. Seven such matings were made and these produced 395 young, consisting of 234 males and 161 females. Details are given below in Table III.

Table III.

Sex-ratio in polygynous mice.

No. of mating	No. of litters	Total young	Males	Females
P. 1	9	59	37	22
P. 2	11	66	33	33
P. 3	8	43	25	18
P. 4	10	66	44	22
P. 5	6	45	26	19
P. 6	10	71	42	29
P. 7	8	45	27	18
Total	62	395	234	161

This gives a male percentage of $59{\cdot}2 \pm 1{\cdot}67$.

The difference between the two percentages is 6·0 and the error on this difference will be:

$$\sqrt{(1{\cdot}67)^2 + ({\cdot}81)^2} = 1{\cdot}85.$$

Hence the difference, $6{\cdot}0 \pm 1{\cdot}85$, is more than three times its probable error, and is therefore statistically significant.

It is clear, however, that certain of these litters, *i.e.* those conceived first in each mating, will not be subject to the influence of increased sexual activity on the part of the male. It would be expected, therefore, that the last few litters born in each mating would show most strongly any effect of polygyny. Unfortunately in only matings P. 6 and P. 7 were records kept of sequence of births, but in these cases, during the last four days in which births occurred, 55 males and 29 females were produced, a ratio of 65·5. No definite statement can be made from

[1] In biological papers the proportion of males is usually expressed as males per 100 females, but this system has the disadvantage that no reliable probable error can be calculated for the ratio. In this paper the percentage of males is used, for which the probable error can be calculated from the formula $\cdot 6745 \sqrt{\frac{M \times F}{N}}$, where m = percentage of males, f = percentage of females, and n the number of individuals.

these small numbers, but they suggest that the effect of polygyny would be more strikingly shown if the dilution caused by the preliminary monogynous litters could be eliminated.

Nevertheless, the experimental evidence as it stands tends to show that polygyny raises the percentage of males.

Discussion.

There are thus three groups of facts which bear upon the effect of polygyny on the sex-ratio, (1) anthropological records of polygynous human races, (2) agricultural data of the amount of stud work allowed to stallions, (3) experimental work on the mouse. Of these, the first material is somewhat contradictory, though in some cases, at least, tending to show that polygyny raises the proportion of males. The other two groups of material, however, seem to show fairly substantially that in other mammals this result is definitely produced.

The explanation of this fact must be sought in one or both of the two factors which govern the sex-ratio at birth. It has been shown elsewhere (Parkes (9)) that in the mouse, as in other mammals, the proportions of the sexes at birth are governed by two factors and that these are:

(*a*) The ratio at conception.

(*b*) The amount and sex-incidence of pre-natal mortality.

It would not appear that the latter of these two could have much bearing on the problem under review. The amount of pre-natal mortality is primarily dependent on factors concerning the mother, and the females used in these experiments were perfectly normal except as regards the fact of there being several in one cage. It has, however, been shown by Hammond (4) that on occasion excessive foetal mortality may be traced to debility of the male. If, however, the possible debility of high polygynous males had the effect of raising the pre-natal mortality it would be expected that (*a*) the size of litter would decrease, (*b*) the proportion of males would decrease[1]. In a previous paper (Parkes (10)) it has been shown that the fertility of polygynous mating is slightly less than that of monogynous ones, but, as regards the other point, we are dealing here with an increase in the proportion of males, not with a decrease.

It would appear, therefore, that the origin of the increase of males must be sought in an increase of males at conception, and, since the

[1] It seems fairly certain that in mammals pre-natal mortality falls more severely upon the males than on the females. In mice there is little doubt that this is so (Parkes, 9). In certain cases, it was found possible to demonstrate an inverse correlation between the amount of pre-natal mortality, and the proportion of males at birth.

amount of foetal mortality is perhaps slightly greater[1], this increase is perhaps more than appears at birth. Since, also, even in the normal matings there is an excess of males at birth, there must be a quite considerable excess at conception, and this in itself needs explanation. As mentioned in a previous paper, the chromosome theory of sex determination, which is now generally accepted, implies that following the production of equal numbers of male and female-producing spermatozoa, the sexes would be conceived in equal numbers. Since this is obviously not so, it must be supposed that for some reason or other one type of spermatozoa, namely the male-producing ones, on the whole survive better the vigorous competition which precedes fertilisation. The presence or absence of the accessory chromosome may provide a basis for the selective action of environmental factors, and since the male-producing type appear to have a smaller head size (Parkes (8)) they may possess some advantage in rapidity of locomotion. However, whatever the exact reason, it does seem that where the spermatozoa are matured under more favourable conditions, the proportion of females increases, whereas when they are matured under less favourable conditions the male proportion goes up. Thus at the height of the breeding season a greater proportion of females is conceived than at times less favourable to reproduction (for fuller account see Parkes (9)).

The explanation of the results of polygyny may, I think, be considered as an extension of this concept. Lloyd-Jones and Hays (5) have shown that the semen of male rabbits subjected to excessive sexual activity becomes extremely debilitated, and, if deduction may be made from other facts, this condition of strained production probably reacts less unfavourably upon the male-producing than upon the female-producing spermatozoa. In such circumstances the observed results of polygyny would be produced, and while such an explanation cannot be regarded as anything more than tentative, it may serve as a first working hypothesis.

The expenses of animal maintenance, etc., were partially defrayed by a grant from the Government Grants Committee of the Royal Society, to whom my best thanks are due.

[1] Probably due to the less favourable conditions (competition, etc.) obtaining where a number of animals are living in one cage.

Summary.

(1) The evidence relating to the effect of polygyny on the sex-ratio in man is conflicting, but the result, if any, appears to be a rise in the proportion of males.

(2) In horses Düsing has shown that the proportion of males rises with increased stud work.

(3) The experimental work on mice described in this paper shows that in this mammal a rise in the percentage of males follows polygynous mating, and therefore supports Düsing's results.

REFERENCES.

(1) Campbell (1870). On Polygyamy—its influence in determining the sex of our Race and its effects on the growth of Population. *Journ. Anthropology*, viii.

(2) Düsing (1884). Die Regulierung des Geschlechtverhältnisses bei der Vermehrung, etc. *Jenaische Zeitsch.* xv.

(3) —— (1887). Die Regulierung des Geschlechtverhältnisses bei Pferden. *Landw. Jahrbucher.* xvi.

(4) Hammond (1921). Factors Controlling Fertility and Foetal Atrophy. *Journ. Agric. Sci.* xi.

(5) Lloyd-Jones and Hays (1918). The Influence of Excessive Sexual Activity of Male Rabbits, I and II. *Journ. of Exp. Zool.* xxv.

(6) Montesquieu (1753). *De L'esprit des Loix*, 3 vols. Genève.

(7) Newcomb (1904). A Statistical Inquiry into the Probability of Causes of the Production of Sex in Human Offspring. *Carnegie Institution Publications*, Washington.

(8) Parkes (1923). Head length Dimorphism of Mammalian Spermatozoa. *Q.J.M.S.* lxvii.

(9) —— (1924). Studies on the Sex-ratio and related Phenomena. (5) The Sex-ratio in Mice and its variations. *Brit. Journ. Exp. Biol.* i.

(10) —— (1924). Fertility in Mice. *Brit. Journ. Exp. Biol.* ii.

(11) Sanderson (1879). Polygamous Marriages among the Kaffirs of Natal and countries around. *Journ. Anthr. Inst.* viii.

(12) Thomas (1913). *Report on the Ibo-speaking peoples of Nigeria*, Pt. 1.

(*Received January 5th*, 1925.)

STUDIES ON CONTACT INSECTICIDES

PART III. A QUANTITATIVE EXAMINATION OF THE INSECTICIDAL ACTION OF THE CHLOR-, NITRO- AND HYDROXYL DERIVATIVES OF BENZENE AND NAPHTHALENE

By F. TATTERSFIELD, B.Sc., F.I.C., C. T. GIMINGHAM, F.I.C.
(*Department of Insecticides and Fungicides*),
and H. M. MORRIS, M.Sc.
(*Department of Entomology*),
Rothamsted Experimental Station.

(With 12 Text-figures.)

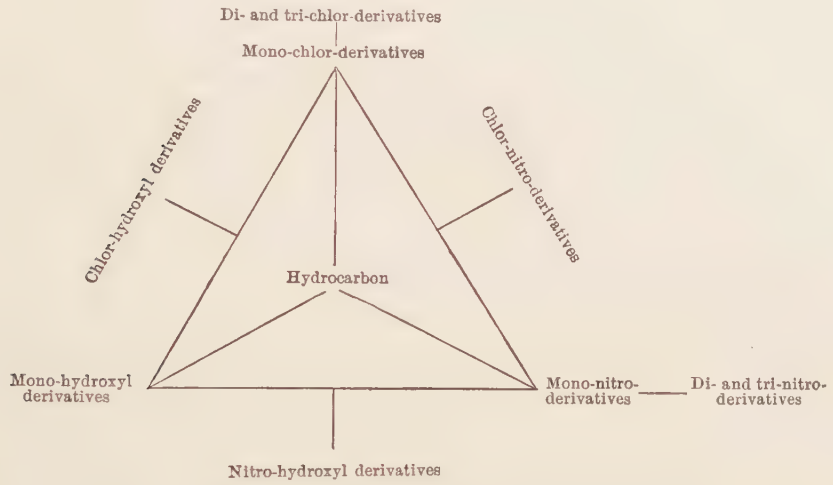
TABLE OF CONTENTS

In Part I of a previous paper (1) we have outlined our objects in attempting to make a preliminary survey of the toxicity to *Aphis rumicis* L. of a number of chemical compounds and plant products. In the present paper we deal with the general results obtained with a group of inter-related substances which are all derivatives of the aromatic hydrocarbons, benzene, toluene and naphthalene. Regarding the hydrocarbons as the parent bodies of the various derivatives we have attempted to follow the effect of the substitution in these compounds of different chemical groups. In dealing with so large a number of different substances, it has not been possible, owing to lack of time and the difficulties of rearing a sufficiently large number of insects, to obtain quantitative results of

a high degree of accuracy with every substance tested; but the results give definite indications of relationships between chemical constitution and toxicity to insects in certain groups of compounds, which can be followed up in further work. In the case of the more toxic substances, which may possibly be of interest from the practical point of view, we have made a larger number of experiments and can claim a higher degree of accuracy for our figures.

The compounds discussed here include one or two which are already known to be toxic to insects and which have been used to some extent in practice. A survey of the kind we have attempted makes it possible to place such derivatives in their correct perspective with regard to allied compounds.

The following scheme gives a diagrammatic view of the relationships between the various types of compounds studied.



We have made such inter-relationships in chemical constitution the basis of our work, and if the tables and diagrams which follow are read with this arrangement in mind, it will be seen that fairly definite indications are obtained of the toxic significance of certain radicals when substituted in the aromatic nucleus. The above diagram does not bring out the importance of the position of substitution in the nucleus but it is hoped that the inclusion of structural formulae in the tables will make this clear. Some of the rarer and, from the practical point of view, less important compounds have been used in only one series of tests, and in some cases differences in toxicity between certain allied compounds were

not great enough to be considered significant without further investigation.

Aphis rumicis L. (adult apterous agamic females) has been used as the chief test insect in order to have a definite standard of comparison throughout; and with all compounds which appeared likely to be of practical importance, we have included experiments on the effect upon various types of foliage. Among the substances considered in this paper, there are several having considerable toxicity to aphides which are at the same time highly injurious to the green parts of plants and which could not therefore, even if otherwise suitable, be considered for use as spray fluids in summer. Such materials might however have a use as winter washes on fruit trees in a dormant condition, and with this point in view, we have made a number of tests on insect eggs. The relative toxic effects of groups of allied compounds were also determined upon eggs for comparison with the results obtained with the same groups of derivatives upon aphides; and there was in general a close correlation between the two sets of results although as would be expected the eggs proved more resistant. The technique employed in connection with tests on eggs differed very little from that used with aphides and is referred to in the section dealing with the results of the experiments.

The experimental data are given both in tables and diagrams. It should be pointed out that the curves on the diagrams are not suitable for mathematical analysis and cannot be used for exact interpolation; they are intended to show the general trend of the experimentally determined points and thus to indicate at a glance, with approximate accuracy, the relative toxicities of the compounds concerned. Where the differences are not significant, a single line on the diagram is used to represent the toxicities of more than one compound.

Experiments with Aphis rumicis *L.*

The method adopted for the preparation of the spraying mixtures, the technique of spraying as carried out by means of the Tattersfield-Morris apparatus and the mode of expressing the results have already been described in previous papers (1), (2) and need not be dealt with here.

A large number of control tests have been made with 1 per cent. saponin solution and with the solvents used. With the great majority of the substances tested, benzene alone proved a satisfactory solvent but in exceptional cases mixtures of benzene with alcohol or with alcohol and ether were employed. Control tests invariably included concentrations of solvent considerably greater than those used in the actual

experiment. The average "mortality figure" (percentage of moribund and dead insects) for 1 per cent. saponin solution was 4 per cent., for benzene 8 per cent.[1], and for the mixtures of solvents 7·5 per cent. These figures are well within the limits of error of our method and, accordingly, have not been taken into account in the tables and diagrams.

Benzene and its Chlor- and Nitro-derivatives.

The results of experiments with benzene, toluene, xylene and some nitro- and chlor-derivatives of these hydrocarbons are given in Table I.

Concentrations are expressed both in grammes and in gramme-molecules (moles.) per 100 c.c. of spray fluid and can be compared with the corresponding percentages of moribund and dead. It will be seen that benzene has no significant toxicity to *A. rumicis* even at very high concentrations; toluene is definitely toxic above 25 per cent. and xylene slightly more toxic than toluene. At the high concentrations (25 and 50 per cent.) the emulsions had an almost gelatinous consistency and some difficulty was experienced in spraying them satisfactorily and this may have influenced the toxic effect.

The introduction of a nitro-group into the benzene ring increases the toxicity to a very considerable degree and a second nitro-group still further accentuates it, m-dinitro-benzene being a highly toxic compound.

The products arising out of the substitution of one chlorine atom into benzene and toluene also show an increased toxicity over the parent bodies, and a progressive increase is noted on passing from monochlor-benzene to the dichlor- and trichlor-derivatives. In these derivatives a pronounced anaesthetic effect was observed, which in the case of p-dichlor-benzene and o-, m-, and p-chlor-toluenes was so marked as to render a numerical expression of the results misleading. With most substances the tendency for moribund insects to die warrants their classification with the dead, but in these cases the tendency is towards recovery and as it was impossible to distinguish between profound anaesthesis and a moribund condition we have preferred in the above four cases merely to give a rough estimate of their toxic properties. It is of interest to note that o-dichlor-benzene is more poisonous than its isomer p-dichlor-benzene.

The order of toxicity of this series of compounds would run:

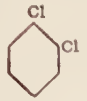
Benzene < toluene < xylene < monochlor-benzene, < p-dichlor-benzene < o-dichlor-benzene, < trichlor-benzene < nitro-benzene < m-dinitro-benzene.

[1] If the figures obtained with benzene which are given in Table I, and which are additional to the regular controls, are also included, the average mortality figure for benzene becomes about 6 per cent.

Table I. *Toxicities to* A. rumicis *of the Aromatic Hydrocarbons and their Chlor- and Nitro-derivatives.*

[N = not affected. S = slightly affected. M = moribund. D = dead.]

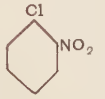
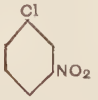
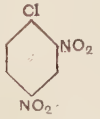
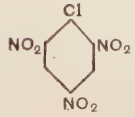
Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
Benzene		5·0	·064	9	1	—	—	0	
M. W. 78		4·0	·051	10	—	—	—	0	
		3·0	·038	8	—	—	2	20	
		2·0	·025	9	—	—	1	10	
		5·0	·064	9	1	—	—	0	
		3·0	·038	9	—	—	1	10	
		1·0	·0128	10	—	—	—	0	
		50·0	·64	10	—	—	—	0	
		25·0	·32	9	—	—	1	10	
		15·0	·192	8	1	—	1	10	
		10·0	·128	9	—	—	1	10	
Toluene		50·0	·54	—	—	—	10	100	
M. W. 92		25·0	·27	5	—	—	5	50	
		15·0	·16	5	1	—	3	33	
		10·0	·11	8	—	1	—	11	
Xylene	$(CH_3)_2:C_6H_4$	50·0	·47	—	—	—	10	100	
M. W. 106		25·0	·235	—	—	3	7	100	
		15·0	·14	1	2	5	2	70	
		10·0	·09	3	—	4	3	70	
Nitro-benzene		5·0	·04	—	—	—	10	100	
M. W. 123		2·5	·02	—	—	—	10	100	
		1·0	·008	—	—	7	3	100	
		0·75	·006	—	—	—	10	100	
		0·5	·004	—	1	4	5	90	
		0·25	·002	4	1	5	—	50	
m-Dinitro-benzene*		0·25	·0015	—	0·3	5·0	4·7	97	Average of several sets of tests.
M. W. 168		0·1	·0006	1·3	—	3·7	5·0	87	
		0·075	·00045	1·5	—	3·5	5·0	85	
		0·05	·0003	4·0	—	2·0	4·0	60	
		0·025	·00015	9·0	—	—	1·0	10	

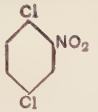
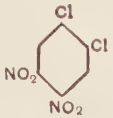
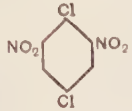
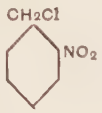
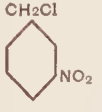
Monochlor-benzene M. W. 112		5·0	·0446	4	—	1	5	60	
		2·5	·022	8	2	—	—	0	
		1·0	·009	10	—	—	—	0	
o-Dichlor-benzene M. W. 147		5·0	·034	—	—	4	6	100	
		4·0	·028	—	—	2	8	100	
		3·0	·021	1	—	6	3	90	
		2·0	·014	2	5	3	—	30	
		1·0	·007	7	2	1	—	10	
p-Dichlor-benzene M. W. 147		5·0	·034	6	2	2	—	20	Figures very irregular, probably owing to anaesthetic action.
		4·0	·028	5	—	5	—	50	
		3·0	·021	5	—	5	—	50	
		2·0	·014	8	—	1	1	20	
		1·0	·007	10	—	—	—	0	
Trichlor-benzene M. W. 181·4	$C_6H_3Cl_3$	0·75	—	—	—	2	8	100	This compound probably contained higher chlorinated derivatives.
		0·5	—	3	—	7	—	70	
		0·25	—	5	—	2	3	50	
		0·1	—	6	—	1	3	40	
o-Chlor-toluene M. W. 126·5									Data unsatisfactory, probably owing to anaesthetic action.
m-Chlor-toluene M. W. 126·5									ditto ditto
p-Chlor-toluene M. W. 126·5									ditto ditto

* The figures in columns N, S, M, D are brought to a basis of ten.

Table I (*continued*)

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
o-Chlor-nitro-benzene M. W. 157·5		5·0	·0317	—	—	—	10	100	
		2·5	·0159	—	—	—	10	100	
		1·0	·0064	—	—	—	10	100	
		0·5	·0032	1	1	2	6	80	
		0·25	·0016	7	—	—	3	30	
m-Chlor-nitro-benzene M. W. 157·5		5·0	·0317	—	—	—	10	100	
		2·5	·0159	—	—	—	10	100	
		1·0	·0064	—	—	—	10	100	
		0·5	·0032	4	1	—	5	50	
		0·25	·0016	7	—	1	2	30	
p-Chlor-nitro-benzene M. W. 157·5		1·0	·0064	—	—	—	10	100	
		0·5	·0032	1	—	3	6	90	
		0·25	·0016	8	—	—	2	20	
1-Chlor-2 : 4-dinitro-benzene* M. W. 202·5		1·0	·00495	—	—	1	9	100	Average of several sets of tests.
		0·75	·0037	—	0·3	0·3	9·4	97	
		0·5	·00247	0·75	—	1·5	7·75	92·5	
		0·4	·00198	0·5	0·5	1	8	90	
		0·3	·00149	4·0	—	2·0	4·0	60	
		0·25	·0012	3·0	0·5	—	6·5	65	
Picryl chloride M. W 247·5									Not appreciably toxic at or below 1 %.

1 : 4-Dichlor-2-nitro-benzene M. W. 192		2·0	·0104	—	—	1	9	100	
		1·0	·005	—	—	1	9	100	
		0·5	·0025	2	—	4	4	80	
		0·25	·00125	8	1	—	1	10	
		0·1	·0005	7	1	1	1	20	
1 : 2-Dichlor-4 : 5-dinitro-benzene M. W. 237									Not appreciably toxic at or below 1 %.
1 : 4-Dichlor-2 : 6-dinitro-benzene M. W. 237									ditto ditto
Benzyl-chloride M. W. 126·5		1·0	·0079	10	—	—	—	0	
		0·75	·0059	10	—	—	—	0	
		0·5	·0039	10	—	—	—	0	
		0·25	·0019	10	—	—	—	0	
		0·1	·00079	9	—	1	—	10	
		0·05	·00039	10	—	—	—	0	
o-Nitro-benzyl-chloride M. W. 171·5		1·0	·0058	—	—	2	8	100	
		0·75	·00435	—	—	3	7	100	
		0·5	·0029	9	—	—	1	10	
		0·25	·00145	7	2	1	—	10	
		0·1	·00058	8	1	—	1	10	
		0·05	·00029	10	—	—	—	0	
m-Nitro-benzyl-chloride M. W. 171·5		1·0	·0058	—	—	2	8	100	
		0·75	·00435	—	—	6	4	100	
		0·5	·0029	4	—	4	2	60	
		0·25	·00145	6	2	1	1	20	
		0·1	·00058	9	—	—	1	10	
		0·05	·00029	10	—	—	—	0	
p-Nitro-benzyl-chloride M. W. 171·5		1·0	·0058	7	1	2	—	20	
		0·75	·00435	9	—	1	—	10	
		0·5	·0029	9	1	—	—	0	
		0·25	·00145	9	1	—	—	0	
		0·1	·00058	10	—	—	—	0	
		0·05	·00029	10	—	—	—	0	

* The figures in columns N, S, M, D are brought to a basis of ten.

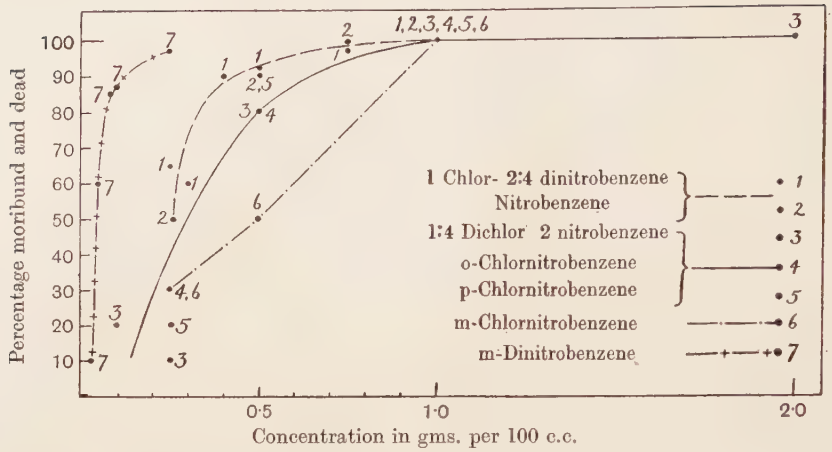


Diagram 1. Showing toxicities of chlor- and nitro-derivatives of benzene to *Aphis rumicis*.

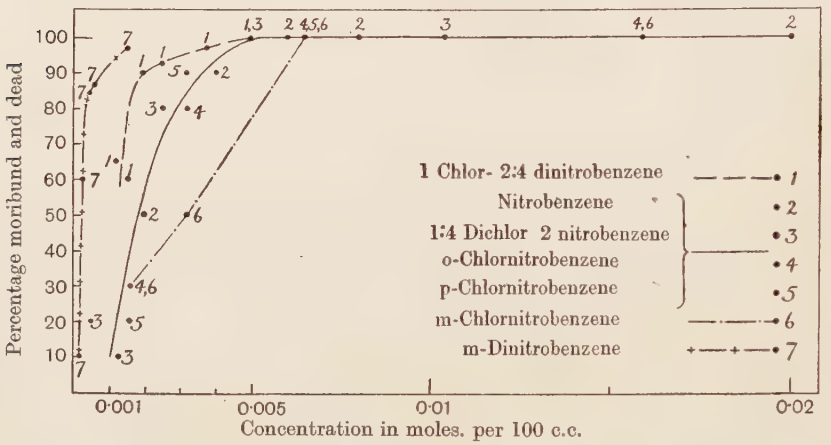


Diagram 2. Showing toxicities of chlor- and nitro-derivatives of benzene to *Aphis rumicis*.

The toxicity of nitro- and chlor-derivatives to insect eggs is discussed on p. 254.

In Table I and Diagrams 1 and 2 the results obtained with compounds containing both chlorine and nitro-groups are also set out. Our experiments do not bring out any significant difference in toxicity between the three isomers, o-, m- and p-chlor-nitro-benzene; these are not appreciably more toxic than nitro-benzene. The introduction of a second nitro-group to form 1-chlor-2: 4-dinitro-benzene increases the poisonous properties but this compound is apparently slightly less toxic than the

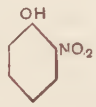
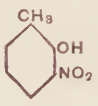
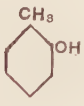
corresponding dinitro-benzene. The presence of a third nitro-group, as in picryl chloride, brings about a marked decrease and this compound has no significant toxicity below 1 per cent. Picryl chloride hydrolyses readily in the presence of water to form picric acid and the composition of the spraying mixture was therefore somewhat uncertain but, as will be shown later, we do not find picric acid itself to have a high toxicity. The influence of the presence of a third nitro-group will be further discussed in connection with the nitro-phenols and cresols.

Of the dichlor-dinitro-derivatives only two were available and these were non-toxic at 1 per cent. and below.

Benzyl chloride is unexpectedly low in toxicity, possibly due to the readiness with which it undergoes chemical change. The nitro-benzyl chlorides are more toxic, the effect of the introduction of a nitro-group depending to some extent on the position of substitution. The order of toxicity in our experiments is benzyl chloride < p-nitro- < o-nitro- < m-nitro-benzyl chloride but the difference between the last two is hardly significant.

Phenols, Cresols and their Chlor- and Nitro-derivatives.

The results obtained for these compounds are shown in Table II and Diagrams 3, 4, 5, 6. Phenol and the three cresols differ very slightly from each other in toxicity and their effectiveness against the aphides is low.

We have given in some detail the results bearing on the effect of introducing nitro-groups in this series of compounds, and some interesting relationships are indicated. Toxicity is not appreciably altered by the introduction of a nitro-group into the o-position to the hydroxyl group of phenol and o-cresol, thus  does not differ significantly from  ; or  from  .

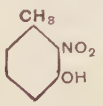
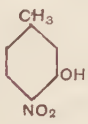
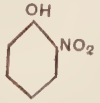
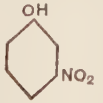
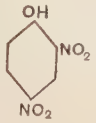
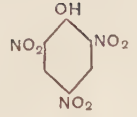
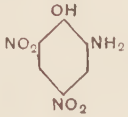
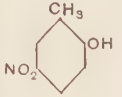
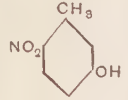
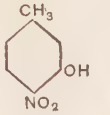
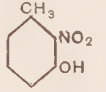
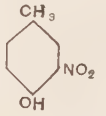
In the case of m-cresol there are two positions having an ortho-relationship to the hydroxyl group, the two compounds having the formulae  and  ; the first of these would appear to be

Table II. *Toxicities to* A. rumicis *of Phenols, Cresols and their Chlor- and Nitro-derivatives.*

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
Phenol M. W. 94		5·0	·0532	—	—	—	10	100	
		2·5	·0266	1	—	6	3	90	
		1·0	·0106	10	—	—	—	0	
		0·5	·0053	10	—	—	—	0	
o-Nitro-phenol M. W. 139		5·0	·036	4	—	—	6	60?	
		2·5	·018	—	—	7	3	100	
		1·0	·0072	9	—	—	1	10	
		0·5	·0036	9	—	—	1	10	
m-Nitro-phenol M. W. 139		2·0	·0144	—	—	—	10	100	
		1·0	·0072	—	—	6	4	100	
		0·5	·0036	9	—	1	—	10	
p-Nitro-phenol M. W. 139		2·0	·0144	—	—	1	9	100	
		1·0	·0072	—	—	4	6	100	
		0·5	·0036	4	1	4	1	50	
		0·25	·0018	5	—	—	5	50	
		0·1	·00072	8	—	—	2	20	
2 : 4-Dinitro-phenol M. W. 184		1·0	·0054	—	—	—	10	100	
		0·5	·0027	—	—	—	10	100	
		0·25	·0013	—	—	—	10	100	
		0·1	·00054	3	—	2	5	70	
		0·1	·00054	4	—	3	3	60	
		0·05	·00027	6	1	1	2	30	
		0·025	·00013	8	1	—	1	10	

Trinitro-phenol (Picric acid) M. W. 229		1·0	·0044	—	—	5	5	100
		0·5	·0022	5	2	1	2	30
Picramic acid M. W. 199								
5-Nitro-o-cresol M. W. 153		2·0	·013	—	—	—	10	100
		1·0	·0065	—	—	—	10	100
		0·5	·0032	—	—	4	6	100
		0·25	·0016	2	2	5	1	60
6-Nitro-m-cresol M. W. 153								
4-Nitro-m-cresol M. W. 153		2·0	·013	—	1	9	—	90
		1·0	·0065	3	1	5	1	60
		0·5	·0032	8	2	—	—	0
		0·25	·0016	10	—	—	—	0
2-Nitro-m-cresol M. W. 153		2·0	·013	—	—	—	10	100
		1·0	·0065	—	—	1	9	100
		0·5	·0032	3	7	—	—	0
		0·25	·0016	7	3	—	—	0
3-Nitro-p-cresol M. W. 153		2·0	·013	—	—	—	10	100
		1·0	·0065	—	—	4	6	100
		0·5	·0032	5	6	—	—	0
		0·25	·0016	7	2	—	1	10

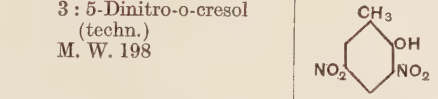
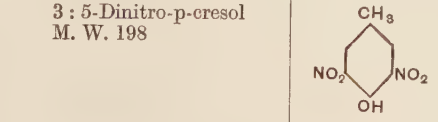
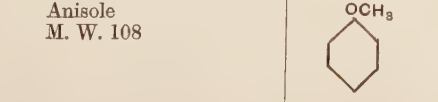
The results of tests with picric acid on different occasions have not been concordant. The figures given are the highest values obtained.

Not appreciably toxic at or below 1 %.

Results unsatisfactory owing to crystallisation from solvent.

Table II (*continued*).

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
3 : 5-Dinitro-o-cresol (techn.) M. W. 198		0·5	·0025	—	—	—	10	100	
		0·25	·00125	—	—	—	10	100	
		0·1	·0005	—	—	—	10	100	
		0·3	·0015	—	—	—	10	100	
		0·2	·0010	—	—	—	10	100	
		0·1	·0005	—	—	—	10	100	
		0·05	·00025	—	2	—	8	80	
		0·025	·000125	8	—	1	1	20	
3 : 5-Dinitro-o-cresol (pure) M. W. 198		0·2	·001	—	—	—	10	100	
		0·1	·0005	—	—	—	10	100	
		0·05	·00025	3	—	—	7	70	
		0·025	·000125	8	—	1	1	20	
3 : 5-Dinitro-p-cresol M. W. 198		2·0	·01	—	2	1	7	80	
		1·0	·005	3	3	1	3	40	
		0·5	·0025	8	—	—	2	20	
		0·25	·00125	8	1	—	1	10	
Trinitro-m-cresol M. W. 243		1·0	·0041	—	—	—	10	100	
		0·5	·002	3	2	—	5	50	
		0·25	·001	8	—	—	2	20	
		0·1	·00041	10	—	—	—	0	
Anisole M. W. 108		5·0	·046	7	—	3	—	30	
		2·5	·023	9	1	—	—	0	
		1·0	·0092	10	—	—	—	0	
		0·5	·0046	9	1	—	—	0	

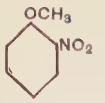
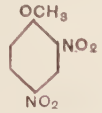
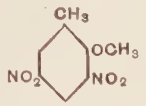
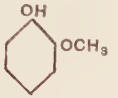
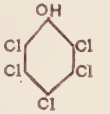
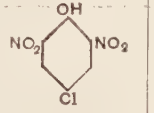
o-Nitro-anisole M. W. 153		2·0	·013	—	—	—	10	100
		1·0	·0065	—	—	—	10	100
		0·5	·0032	2	—	2	6	80
		0·25	·0016	4	—	3	3	60
		0·1	·00065	10	—	—	—	0
		0·05	·00032	10	—	—	—	0
p-Nitro-anisole M. W. 153		2·0	·013	—	—	—	10	100
		1·0	·0065	—	—	—	10	100
		0·5	·0032	1	1	—	8	80
		0·25	·0016	4	—	1	5	60
		0·1	·00065	6	—	2	2	40
		0·05	·00032	8	—	1	1	20
2 : 4-Dinitro-anisole M. W. 198								Not appreciably toxic below 1 %.
3 : 5-Dinitro-o-methoxy-toluene M. W. 212								Not appreciably toxic at or below 1 %.
Guiacol M. W. 124		5·0	·0403	—	—	—	10	100
		2·5	·0201	7	—	—	3	30
		1·0	·008	7	—	2	—	22·2
		0·5	·004	9	—	1	—	10
		0·25	·002	8	—	—	2	20
Pentachlor-phenol M. W. 266		1·0	·0376	—	—	4	6	100
		0·75	·0282	—	—	5	5	100
		0·5	·0188	3	1	—	6	60
		0·25	·0094	3	2	1	4	50
		0·1	·00376	6	—	—	4	40
		0·05	·00188	6	—	—	1	14·3
Chlor-dinitro-phenol M. W. 218·5		1·0	·00457	—	—	—	10	100
		0·75	·0034	—	—	—	10	100
		0·5	·00228	—	—	1	9	100
		0·25	·0011	4	—	2	4	60
		0·1	·00045	7	1	—	2	20
		0·05	·00023	10	—	—	—	0
		0·025	·00011	10	—	—	—	0

Table II (*continued*).

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
Dichlor-cresol (crude) M. W. 177		1·0	—	—	—	5	5	100	
		0·75	—	—	—	5	5	100	
		0·5	—	10	—	—	—	0	
		0·25	—	10	—	—	—	0	
o-Cresol M. W. 108		5·0	·0463	—	—	—	10	100	
		2·5	·0231	—	—	—	10	100	
		1·0	·0092	9	—	—	1	10	
		0·5	·0046	10	—	—	—	0	
m-Cresol M. W. 108		5·0	·0463	—	—	—	10	100	
		2·5	·0231	—	—	2	8	100	
		1·0	·0092	—	1	4	1	83·3	
		0·5	·0046	10	—	—	—	0	
p-Cresol M. W. 108		5·0	·0463	—	—	—	10	100	
		2·5	·0231	1	—	2	7	90	
		1·0	·0092	2	3	4	1	50	
		0·5	·0046	9	1	—	—	0	
3-Nitro-o-cresol M. W. 153		2·0	·0130	4	2	4	—	40	
		1·0	·0065	7	—	3	—	30	
		0·5	·00325	10	—	—	—	0	
		0·25	·0016	8	2	—	—	0	

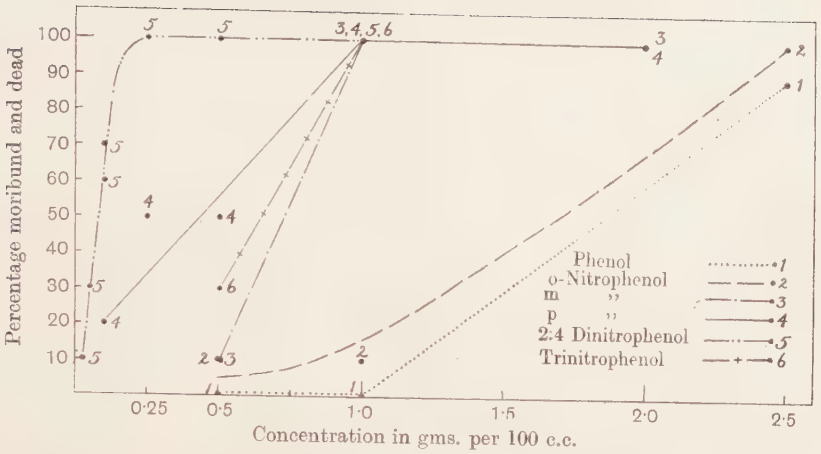


Diagram 3. Showing toxicities of phenol and its nitro-derivatives to *Aphis rumicis*.

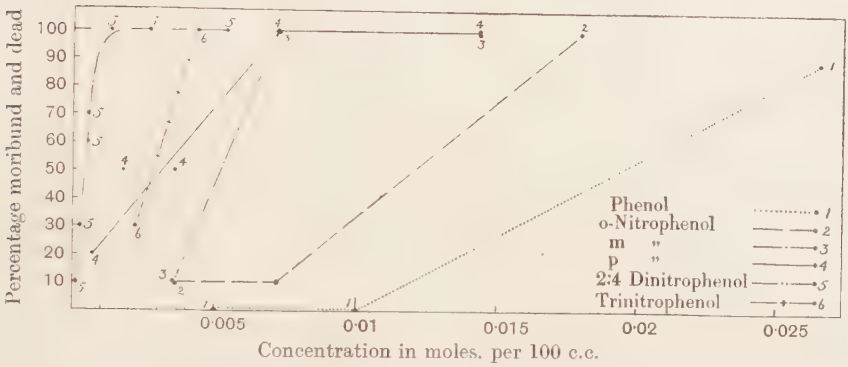


Diagram 4. Showing toxicities of phenol and its nitro-derivatives to *Aphis rumicis*.

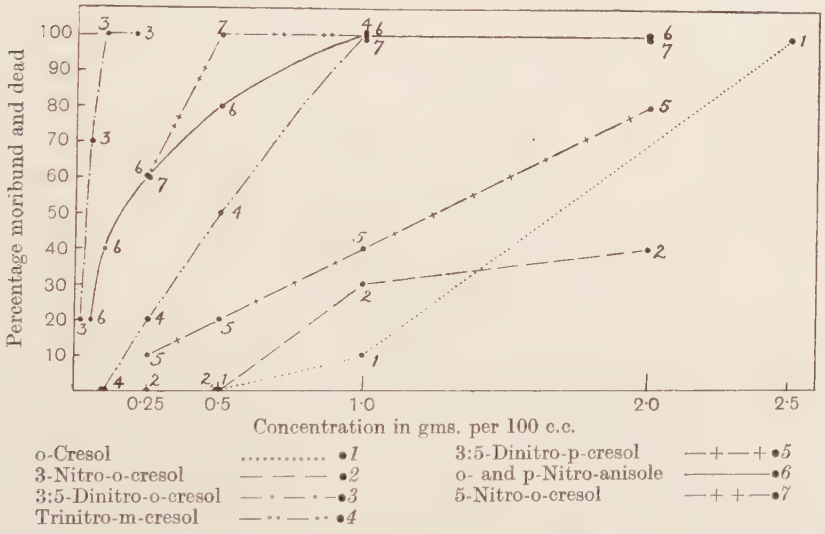


Diagram 5. Showing toxicities of nitro-derivatives of cresol and anisole to *Aphis rumicis*.

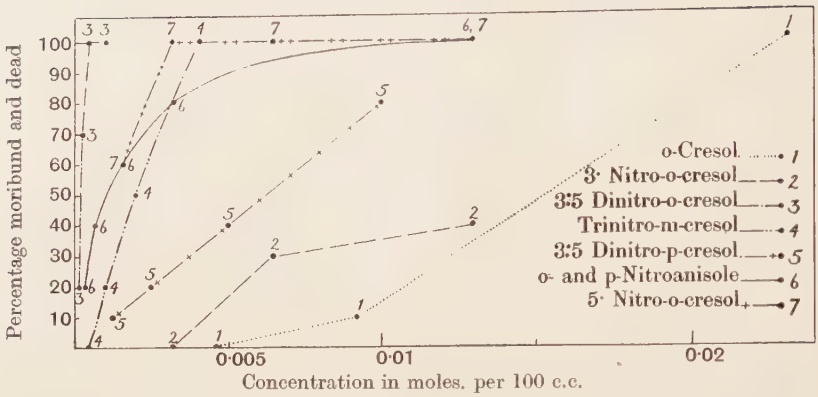
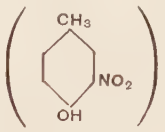
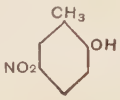
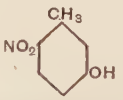
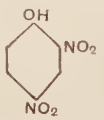


Diagram 6. Showing toxicities of nitro-derivatives of cresol and anisole to *Aphis rumicis*.

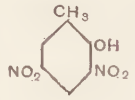
slightly more toxic than the other but in neither of these cases nor in that of 3-nitro-p-cresol  does the introduction of the nitro-group in this position cause any very large increase in toxic properties, a result which was not expected in view of the effect of introducing this group into benzene.

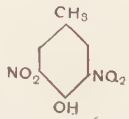
The introduction of the nitro-group into the para-position to the hydroxyl group however, in the case of phenol and o-cresol, to form the compounds  and  leads to a material increase in the toxic value. The corresponding nitro-compound of m-cresol  gave irregular results owing to the difficulties encountered in preparing a satisfactory spraying mixture. m-nitro-phenol was rather more toxic than the o-compounds but very slightly less toxic than p-nitro-phenol.

The following dinitro-derivatives were tested:

(*a*) 2 : 4-dinitro-phenol[1]..... 

[1] Some difficulty was experienced in making a satisfactory spraying mixture with 2 : 4 dinitro-phenol owing to its slight solubility in benzene and it was necessary to make use of a mixture of ether, alcohol and benzene. As already mentioned, control experiments with this mixture were quite satisfactory and the results obtained are comparable with those from the other two dinitro-derivatives.

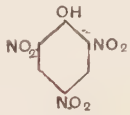
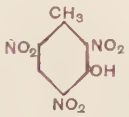
(*b*) 3 : 5-dinitro-o-cresol..... 

and (*c*) 3 : 5-dinitro-p-cresol..... 

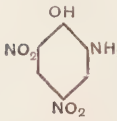
Nos. (*a*) and (*b*) above are analogues in chemical structure, one nitro-group of each compound being in the o-position to the hydroxyl group and the other in the p-position, while in No. (*c*) the two groups occupy two o-positions to the hydroxyl group.

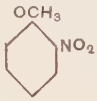
Both 2 : 4-dinitro-phenol and 3 : 5-dinitro-o-cresol proved highly toxic to the aphides, the cresol-derivative, both weight for weight and mole for mole, being somewhat the more toxic of the two. As is well known, the potassium salt of 3 : 5-dinitro-o-cresol has been used as an insecticide. We have tested this and other salts on insect eggs (see p. 256) and these and other compounds which present interest from a practical point of view are discussed on p. 257.

Dinitro-p-cresol (*c*) was much less toxic than the other two dinitro-derivatives (*a*) and (*b*), suggesting as in the mono-derivatives that substitution of the nitro-group in the o-position to hydroxyl has not the toxic significance that it possesses when in the p-position to that group.

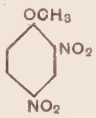
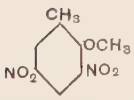
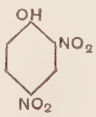
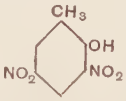
The introduction of a third nitro-group lowers the toxicity materially, the two trinitro-derivatives tested, namely picric acid  and trinitro-meta-cresol  which are analogues, both proving to be much less effective than 2 : 4-dinitro-phenol and 3 : 5-dinitro-o-cresol. There are no exactly analogous compounds in the case of the o- and p-cresols.

Summarising the position with respect to these compounds, it appears to be established that their toxicity runs in the following order: Phenol < o-nitro-phenol < m- and p-nitro-phenol < 2 : 4-dinitro-phenol > trinitro-phenol (picric acid) and the same order would apply to the cresols and their corresponding derivatives.

Picramic acid  was also tested, but this compound was found to have practically no toxic effect at or below a concentration of 1 per cent.

Anisole and some of its derivatives were also tested in order to follow the effect of the methylation of the hydroxyl group. Anisole is rather less toxic than phenol but o-nitro-anisole  is more so than its isomer 3-nitro-o-cresol and than o-nitro-phenol.

o- and p-nitro-anisole have about the same toxicity as 4-nitro-o-cresol.

2 : 4-dinitro-anisole  and 3 : 5-dinitro o-methoxytoluene  were found to be less strikingly poisonous than the corresponding phenol compounds  and  or than the mono-nitro-anisoles. This decrease in toxicity on further nitration was unexpected. In the case of dinitro-anisole, some difficulty was met with in preparing an emulsion suitable for spraying, the material tending to separate out in crystalline form; but this would not seem to account wholly for the difference, as in the experiments where eggs were used (see p. 254) there was the expected increase in toxic action on passing to the dinitro-derivatives. It is possible that dinitro-anisole acts only slowly as a contact poison and that, had we been able to keep the aphides under observation for a longer period, a higher toxicity would have been indicated for this compound. Nevertheless, it is noteworthy that neither dinitro-anisole nor dinitro-o-methoxytoluene showed any pronounced poisonous action to aphides under the conditions of our experiment, whereas the corresponding dinitro-phenol and dinitro-o-cresol showed both rapid and intense toxic action at very low concentrations.

The results obtained with a few chlorine derivatives of the phenol group are also set out in Table II but as far as these were investigated they showed no very marked increases in toxic action over the parent bodies.

Naphthalene Derivatives.

The results obtained for certain derivatives of naphthalene are set down in Table III and Diagrams 7 and 8. These compounds, with the exception of the amines, which will be dealt with in another paper, are collected together here for purpose of convenient reference. The cyanides are included in this table for comparison with α-chlor-naphthalene.

Naphthalene itself did not prove to have toxic properties of a high order; there was however some difficulty in getting it into a suitable form for spraying. The product tetra-hydro-naphthalene (Tetralin) resulting from the partial hydrogenation of naphthalene was not significantly different in toxicity. As this compound presents no difficulty in working up into a form suitable for spraying and is likely to be available in considerable quantities it may possess interest from a practical point of view.

Complete reduction to deca-hydro-naphthalene results in a pronounced loss of toxicity which may perhaps be correlated with the aliphatic properties of this compound.

Only a few of the nitro-naphthalene and naphthol derivatives have so far been tested. α-nitro-naphthalene is fairly toxic at 1 per cent. and may be worth further investigation but 1:8-dinitro-naphthalene is appreciably less toxic than the mono-compound. α-naphthol was not more toxic than the cresols; dinitro-α-naphthol, which by analogy with dinitro-phenol and cresol should have proved a particularly interesting compound did not fulfil expectations. It was very insoluble in organic solvents and not readily worked up into a form suitable for spraying. The salts of this and allied compounds were not tested but may be worth trial. α-chlor-naphthalene proved in our tests to be comparatively highly toxic. Average figures for all the data obtained by us for this compound are expressed in Table III and are plotted on Diagrams 7 and 8.

The two cyanogen derivatives do not differ materially in toxicity molecule for molecule either from one another or from α-chlor-naphthalene, a rather unexpected result.

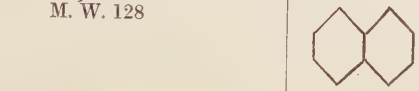
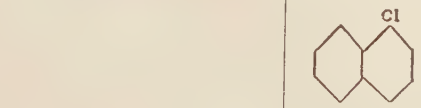
α-chlor-naphthalene was also tried on a larger scale upon aphis-infested bean plants, at a concentration of 0·35 per cent. and killed 99–100 per cent.[1] of the insects.

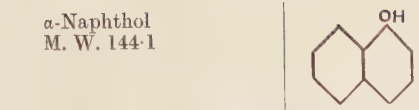
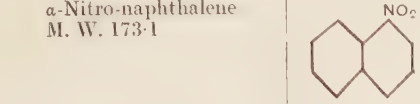
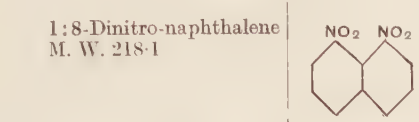
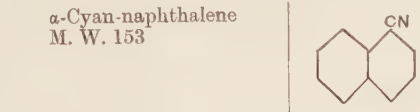
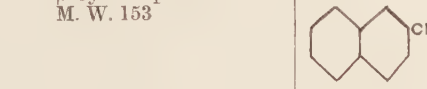
The chlor-benzene derivatives possess anaesthetic properties as already mentioned, which render the determination of their actual toxic values rather uncertain. In the case of α-chlor-naphthalene, anaesthesis

[1] Soft soap in a concentration of 0·5 per cent. was used as an emulsifying and wetting agent in this experiment and itself possessed a moderately high toxicity.

Table III. *Toxicities to* A. rumicis *of various Naphthalene-derivatives.*

[N = not affected. S = slightly affected. M = moribund. D = dead.]

Substance	Formula	Concentration		N	S	M	D	% moribund and dead	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.						
Naphthalene M. W. 128		10·0	·078	—	—	—	9·0	100	Average of two trials.
		5·0	·039	—	—	10·0	—	100	
		2·5	·0195	—	—	10·0	—	100	
		1·0	·0078	3·5	1·0	4·0	1·5	55	
		0·5	·0039	7·0	—	1·0	2·0	30	
		0·25	·0019	8·0	—	—	2·0	20	
Tetra-hydro-naphthalene* M. W. 132		10·0	·0758	—	—	—	10·0	100	Average of several sets of figures.
		5·0	·0379	—	—	—	10·0	100	
		2·5	·0189	1·0	—	2·0	7·0	90	
		1·0	·0076	3·0	1·0	4·7	1·7	64	
		0·5	·0038	6·0	—	1·5	2·5	40	
		0·25	·0019	6	4	—	—	0	
Deca-hydro-naphthalene M. W. 138		10·0	·0725	—	—	—	10	100	
		5·0	·0362	10	—	—	—	0	
		2·5	·0181	8	—	1	1	20	
		1·0	·0072	9	—	—	1	10	
α-chlor-naphthalene* M. W. 162·5		5·0	·0307	—	—	—	10·0	100	Average of several sets of figures.
		2·5	·0153	—	—	—	10·0	100	
		1·0	·0061	—	—	—	10·0	100	
		0·5	·0030	—	—	—	10·0	100	
		0·4	·0024	—	1·0	1·0	8·0	90	
		0·35	·0021	—	—	—	9·0	100	
		0·25	·0015	—	1·0	5·0	4·0	90	
		0·2	·0012	3·0	—	2·0	5·0	70	
		0·1	·0006	4·5	—	3·0	2·5	55	
		0·075	·00046	5·0	—	—	3·0	37·5	
		0·05	·0003	5·0	1·0	0·5	3·0	36·8	

α-Naphthol M. W. 144·1		5·0 2·5 1·0 0·5 0·25	·0347 ·0173 ·0069 ·0034 ·0017	1 2 5 9 10	— — — — —	— 1 — — —	10 8 5 1 —	100 81·8 50 10 0	
Dinitro-α-naphthol M. W. 234·1									Not appreciably toxic below 0·2 %. Difficult to spray.
α-Nitro-naphthalene M. W. 173·1		1·0	—	—	—	10	—	100	
1:8-Dinitro-naphthalene M. W. 218·1		Less than 1·0	—	10	—	—	—	0	The 1 : 5 derivative could not be worked up into a form suitable for spraying.
α-Cyan-naphthalene M. W. 153		1·0 0·5 0·25 0·1 0·05 0·025	·0065 ·0033 ·0016 ·0006 ·0003 ·00016	— — 2 9 10 10	— — — — — —	1 6 8 1 — —	9 4 — — — —	100 100 80 10 0 0	
β-Cyan-naphthalene M. W. 153		1·0 0 5 0·25 0·1 0·05 0·025	·0065 ·0033 ·0016 ·0006 ·0003 ·00016	— — 3 10 9 9	— — — — — —	— 1 2 — 1 1	10 9 5 — — —	100 100 70 0 10 10	

* The figures in columns N, S, M, D are brought to a basis of ten.

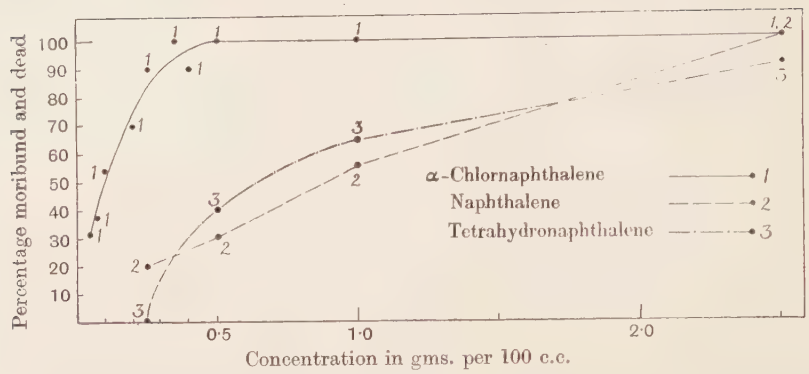


Diagram 7. Showing toxicities of naphthalene derivatives to *Aphis rumicis*.

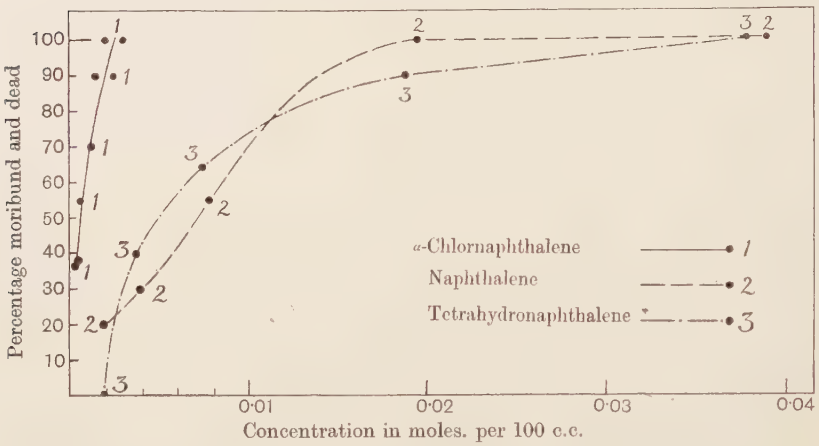


Diagram 8. Showing toxicities of naphthalene derivatives to *Aphis rumicis*.

is not definitely indicated but the chlor-naphthalene group evidently requires further investigation since the possibility of an anaesthetic action cannot be excluded.

Experiments with the eggs of Selenia tetralunaria *Hufn.*

For the experiments with insect eggs, we were able to obtain large numbers of eggs of the Geometrid moth, *Selenia tetralunaria* Hufn., laid on muslin; in the first place by purchase, and a second generation by confining adults in large net bags. The insects were carefully protected in all stages and were completely free from parasites. Small pieces of muslin, each bearing a suitable number of eggs, were cut off, placed in small dishes and sprayed in the machine as in the experiments with aphides. They were then set aside in the insectary in glass dishes covered

with muslin and, when hatching began, the young larvae emerging were counted each day. The larvae of this species do not eat the empty egg-shells and as these remain attached to the muslin and are readily distinguishable from unhatched eggs, it was possible to make a confirmatory count of hatched and unhatched eggs when all hatching was definitely at an end.

Considerable difficulty was met with in making sure that none of the very small newly emerged larvae escaped; and when large numbers of eggs were used the second method of counting was relied upon. The pieces of muslin were numbered and pinned on cards which were placed in large glass vessels, the counting being deferred until no more eggs were observed to hatch.

In the consideration of the results of the experiments with eggs, it was necessary to take into account the figures obtained in the controls since a proportion of the eggs did not hatch[1] irrespective of treatment. Two separate series of tests were carried out; and in each case the appropriate control figure has been allowed for by deducting the average percentage of unhatched eggs in the controls (a) from the percentage unhatched in each test and multiplying by $\frac{100}{100-a}$. The effect of allowing for controls in this manner is relatively less in the case of high than of low mortality figures. If, after spraying, there are 100 per cent. unhatched eggs the calculation leaves this figure unchanged. The effect of the correction progressively increases until the percentage unhatched reaches the control figure at which point (and below) the compound tested is regarded as having no significant toxic action.

The *first series* included tests with 1-chlor-2 : 4-dinitro-benzene, 3 : 5-dinitro-o-cresol (both of these compounds are highly toxic to aphides), picric acid, nicotine and Carbolineum-Krimpen, the well-known proprietary winter spray fluid. The results are given, together with the control figures, in Table IV and plotted in Diagrams 9, 10.

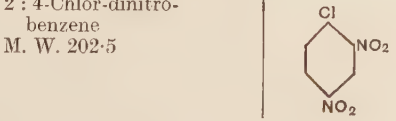
Three sets of controls are given: unsprayed, sprayed with 1 per cent. saponin solution, and sprayed with benzene at various concentrations emulsified in 1 per cent. saponin solution. We have taken the figure 12 per cent. as a fair average for the percentage of eggs not hatching in the controls. The very high figure of 33·3 per cent. unhatched (see Table) given by one batch of unsprayed eggs is obviously abnormal and has been omitted in arriving at the average[2]; the probability of its occurrence

[1] This does not include obviously unfertilised eggs which, in this species, differ in colour from fertilised eggs. These were neglected in all the counts.

[2] Including this figure, the average would have been 14·1 per cent.

Table IV. *Toxicity to the eggs of* Selenia tetralunaria.

[No. of eggs not hatching in controls taken as 12 %.]

Substance	Formula	Concentration		No. of eggs sprayed	% of eggs not hatched	% hatching calc. on control	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.				
Unsprayed control		—	—	21	33·3	—	
		—	—	21	4·75	—	
		—	—	24	12·5	—	Average % not hatching 14·1*.
		—	—	20	15·0	—	
		—	—	20	5·0	—	
Saponin control		1·0	—	23	0	—	
		1·0	—	19	15·75	—	Average % not hatching 10·2*.
		1·0	—	20	15·0	—	
Benzene control		12·5	—	22	13·65	—	
		5·0	—	24	4·16	—	
		2·5	—	20	5·0	—	
		1·25	—	21	14·3	—	Average % not hatching 8·58.
		0·5	—	23	4·35	—	
		0·25	—	20	10·0	—	
2 : 4-Chlor-dinitro-benzene M. W. 202·5		2·5	·012	19	94·5	93·75	
		1·0	·005	21	76·25	73·0	
		0·5	·0025	21	23·8	12·4	
		0·25	·00125	28	0	—	
		0·1	·0005	20	15·0	3·4	
		0·05	·00025	21	14·6	3·2	

3 : 5-Dinitro-o-cresol M. W. 198		2·0	·01	19	100·0	100·0	—
		1·0	·005	23	100·0	100·0	—
		0·5	·0025	20	100·0	100·0	—
		0·2	·001	22	100·0	100·0	—
		0·1	·0005	20	100·0	100·0	—
		0·05	·00025	20	94·7	93·0	—
Picric acid M. W. 229		1·0	·0044	20	25·0	14·75	—
		0·5	·0022	21	23·8	13·4	—
		0·25	·0011	18	16·65	5·3	—
		0·1	·00044	21	19·05	8·0	—
		0·05	·00022	22	9·07	—	—
Carbolineum	—	7·0	—	20	100·0	100·0	—
		5·0	—	21	100·0	100·0	—
		2·5	—	20	20·0	9·1	—
		1·0	—	20	15·0	3·4	—
		0·5	—	20	5·0	—	—
Nicotine	—	1·0	—	20	100·0	100·0	—
		0·5	—	20	100·0	100·0	—
		0·25	—	20	100·0	100·0	—
		0·1	—	20	100·0	100·0	—
		0·05	—	23	91·4	90·2	—
		0·025	—	20	60·0	54·5	—

* Average 12·2.

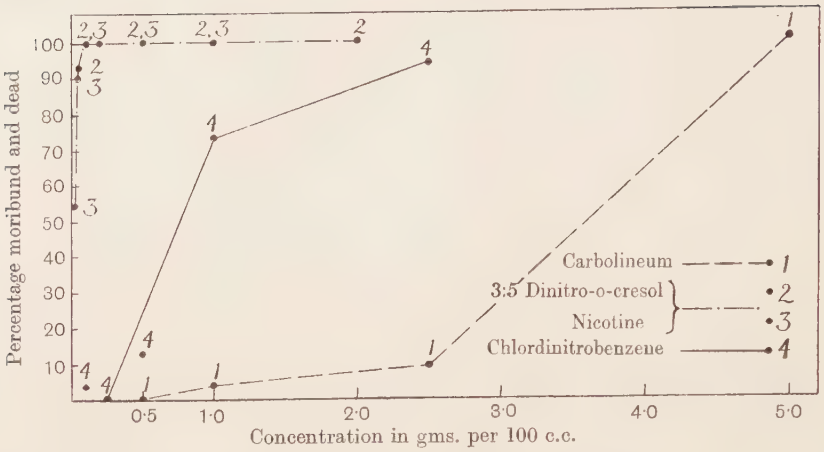


Diagram 9. Showing toxicities of dinitro-o-cresol and other substances to eggs of *Selenia tetralunaria*.

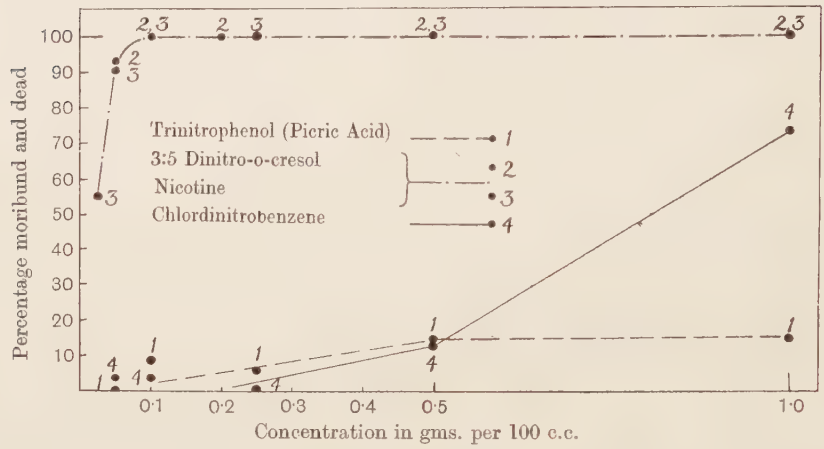


Diagram 10. Showing toxicities of dinitro-o-cresol and other substances to eggs of *Selenia tetralunaria*.

is approximately 1 in 50 trials. Allowance for the control figure of 12 per cent. has been made in the manner explained above and the corrected figures are given in column 7 of Table IV; these values are also used in the diagrams.

The number of eggs used in each test in this series was rather small (about 20) and the results are only approximate; and the curves on the diagrams are not considered to do more than bring into prominence and render more easy a comparison of the relative toxicities of the compounds. The high toxicity of dinitro-o-cresol is very evident; a con-

centration as low as 0·1 per cent. killed 100 per cent. of the eggs of *S. tetralunaria* and the substance appears to be almost as toxic to these eggs as to adult aphides; whereas picric acid (trinitro-phenol) had very little effect, agreeing with its low toxicity to aphides. Nicotine was slightly less toxic than dinitro-o-cresol. Carbolineum, as was expected from its known toxicity to the eggs of aphides and Psyllidae, killed 100 per cent. at a dilution of 5 per cent.

Chlor-dinitro-benzene, although markedly less poisonous than dinitro-o-cresol, may possess interest from a practical point of view as it can be obtained at a relatively low price.

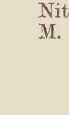
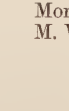
The *second series* of experiments with eggs of *Selenia tetralunaria* was rather more extensive and the compounds tested included a number of groups of closely allied derivatives in order to ascertain whether the relationships between the individual members of such groups as to toxicity to eggs would be in the same order as the toxicity to aphides. In addition, chlor-dinitro-benzene and dinitro-o-cresol were tested again together with some metallic salts of the latter since these are soluble in water and one of them (the potassium salt) has, as already mentioned, been employed as an insecticide on a practical scale. The results of this series of tests are given in Table V and Diagrams 11 and 12.

In this series of experiments, the number of eggs used was generally rather larger than in the first series, though it was not possible to take a constant number for each test owing to uneven distribution on the muslin. The actual average percentage which failed to hatch in the control tests with 1 per cent. saponin and with various concentrations of benzene was 6·2; the figures however varied very widely (from 0–21·4 per cent.) and it was considered wiser to take the round figure of 20 per cent. as the control, in order to make sure of avoiding any risk of exaggerating toxicity. The corrected figures, given in column 7 of Table V, were therefore calculated, in the manner already mentioned, allowing for 20 per cent. of the eggs failing to hatch irrespective of treatment. As a further check on the value of this figure, we submitted to Miss W. A. Mackenzie of the Statistical Department at Rothamsted, all our figures for control experiments and for those tests in which the results gave no significant indication of toxic effect. Miss Mackenzie has analysed these figures and kindly allows us to include the following note:

A series of 59 sprayings (14 series of parallel sprayings), in which there was every reason to believe that no toxic effect was shown, was examined. The averages of the several parallel sprayings showed that 4·9 per cent. to 21·2 per cent. of the eggs failed to hatch, the general mean being 12·1 per cent. The variation within the

Table V. *Toxicity of various compounds to the eggs of* Selenia tetralunaria.

[No. of eggs not hatching in controls taken as 20 %.]

Substance	Formula	Concentration		No. of eggs sprayed	% of eggs not hatching	% hatching calc. on control	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.				
Saponin		1·0	—	27	7·4	—	
		1·0	—	26	7·7	—	
Benzene		15·0 (c.c.)	—	42	21·4	—	
		10·0 ,,	—	19	0	—	
		5·0 ,,	—	31	19·35	—	
Benzene		10·0 ,,	—	25	0	—	
Ether		10·0 ,,	—	—	—	—	
Benzene		5·0 ,,	—	20	0	—	
Ether		5·0 ,,	—	—	—	—	
Benzene		10·0 ,,	—	32	0	—	
Alcohol		10·0 ,,	—	—	—	—	
Ether		10·0 ,,	—	—	—	—	
Benzene		5·0 ,,	—	26	0	—	
Alcohol		5·0 ,,	—	—	—	—	
Ether		5·0 ,,	—	—	—	—	
Nitro-benzene M. W. 123							Not appreciably toxic at or below 5 %.
m-Dinitro-benzene M. W. 168		5·0	·03	33	97·0	96·2	
		2·5	·015	21	76·5	70·6	
		1·0	·006	24	20·8	—	
		0·5	·003	41	2·44	—	
		0·25	·0015	17	11·8	—	
Monochlor-benzene M. W. 112							Not appreciably toxic at or below 5 %.

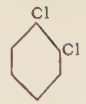
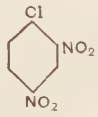
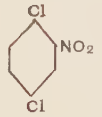
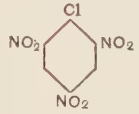
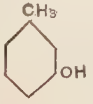
o-Dichlor-benzene M. W. 147							ditto ditto
p-Dichlor-benzene M. W. 147							ditto ditto
Trichlor-benzene		5·0		31	90·4	88·0	Probably contained higher chlorinated derivatives.
		2·5		16	62·2	52·7	
		1·0		21	19·0	—	
		0·5		25	20·0	--	
		0·25		20	25·0	6·25	
o-Chlor-nitro-benzene M. W. 157·5							Not appreciably toxic at or below 5 %.
m-Chlor-nitro-benzene M. W. 157·5							ditto ditto
p-Chlor-nitro-benzene M. W. 157·5							ditto ditto
1-Chlor-2 : 4-dinitro-benzene M. W. 202·5		2·5	·012	68	75·0	68·5	
		1·0	·00495	23	4·35	—	
		0·5	·00247	20	10·0	—	
		0·25	·00123	35	28·6	10·5	
		0·1	·0006	25	4·0	--	

Table V (*continued*).

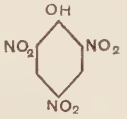
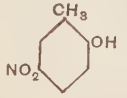
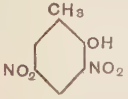
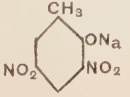
[No. of eggs not hatching in controls taken as 20 %.]

Substance	Formula	Concentration		No. of eggs sprayed	% of eggs not hatching	% hatching calc. on control	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.				
1 : 4-Dichlor-2-nitro-benzene M. W. 192		2·0	·0104	34	52·8	41·0	
		1·0	·0052	38	39·4	24·2	
		0·5	·0026	22	13·6	—	
		0·25	·0013	19	10·6	—	
Picryl chloride M. W. 247·5							Not appreciably toxic at or below 1 %.
Phenol M. W. 94		5·0	·0532	23	13·1	—	
		2·5	·0266	26	19·25	—	
		1·0	·0106	43	18·6	—	
o-Cresol M. W. 108		5·0	·0463	35	11·4	—	
		2·5	·0231	22	27·2	9·0	
		1·0	·0092	34	5·9	—	
m-Cresol M. W. 108		5·0	·0463	25	48·0	35·0	
		2·5	·0231	32	19·0	—	
		1·0	·0092	32	12·5	—	
p-Cresol M. W. 108		5·0	·0463	21	33·3	16·6	
		2·5	·0231	41	22·0	2·5	
		1·0	·0092	48	4·16	—	

Anisole M. W. 108		5·0	·0463	38	42·1	22·2	
		2·5	·0231	26	23·0	3·8	
		1·0	·0092	43	16·3	—	
Xylenol mixture		5·0	—	27	100·0	100·0	
		2·5	—	33	39·4	24·2	
		1·0	—	26	3·85	—	
o-Nitro-phenol M. W. 139		5·0	·036	20	0	—	
		2·5	·018	40	7·5	—	
		1·0	·0072	70	2·87	—	
		0·5	·0036	80	6·25	—	
		0·25	·0018	30	26·7	8·0	
m-Nitro-phenol M. W. 139		2·0	·0144	42	4·75	—	
		1·0	·0072	29	10·33	—	
		0·5	·0036	37	10·8	—	
		0·25	·0018	41	7·3	—	
p-Nitro-phenol M. W. 139		2·0	·0144	47	10·65	—	
		1·0	·0072	62	9·67	—	
		0·5	·0036	43	18·6	—	
		0·25	·0018	49	14·3	—	
2 : 4 Dinitro-phenol M. W. 184		1·0	·0054	38	100·0	100·0	
		0·5	·0027	34	100·0	100·0	
		0·25	·0013	42	83·4	79·0	
		0·1	·00054	30	40·0	25·0	
		0·05	·00027	19	26·3	7·85	
Sodium dinitro-phenate		1·0 D.N.P.	·0054	24	50·0	37·5	Results too irregular to graph.
		0·5	·0027	57	43·0	43·0	
		0·25	·0013	33	18·15	—	
		0·1	·00054	34	23·5	4·35	
		0·05	·00027	31	25·8	7·25	
		0·025	·00013	26	7·7	—	

Table V (*continued*).

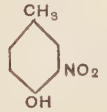
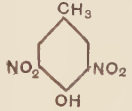
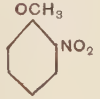
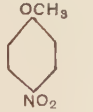
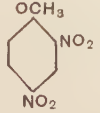
[No. of eggs not hatching in controls taken as 20 %.]

Substance	Formula	Concentration		No. of eggs sprayed	% of eggs not hatching	% not hatching calc. on control	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.				
Picric acid M. W. 229		1·0	·0044	27	3·7	—	
		0·5	·0022	37	16·2	—	
3-Nitro-o-cresol M. W. 153		2·0	·013	46	8·7	—	
		1·0	·0065	26	11·5	—	
		0·5	·00325	28	10·7	—	
		0·25	·0016	27	0	—	
5-Nitro-o-cresol M. W. 153							Toxicity not determined owing to difficulties in spraying.
3 : 5-Dinitro-o-cresol M. W. 198		1·0	·005	38	100·0	100·0	
		0·5	·0025	49	100·0	100·0	
		0·25	·00125	54	100·0	100·0	
		0·1	·0005	42	80·9	76·0	
		0·05	·00025	38	31·6	14·5	
		0·025	·000125	36	19·45	—	
Sodium-dinitro-o-cresylate		1·0 D.N.C.	—	35	91·4	89·2	
		0·5	—	31	80·6	75·7	
		0·25	—	27	77·7	72·0	
		0·1	—	33	63·6	54·5	
		0·05	—	39	23·05	3·8	
		0·025	—	35	14·3	—	

Potassium-dinitro-o-cresylate		1·0 D.N.C.		46	97·8		97·5
		0·5		32	94·0		92·5
		0·25		40	85·0		81·3
		0·1		35	34·3		17·9
		0·05		22	45·5		31·9
		0·025		29	24·2		5·4
Barium-dinitro-o-cresylate		0·33		25	60·0		50·0
		0·1		25	64·0		55·0
		0·05		39	35·9		19·85
		0·025		22	27·2		9·0
3 : 5-Dinitro-o-methoxy-toluene M. W. 212		2·0	·0091	56	68·0		60·0
		1·0	·0047	53	75·5		69·3
6-Nitro-m-cresol M. W. 153		2·0	·013	34	14·7		
		1·0	·0065	33	12·1		
		0·5	·0032	39	10·25		
4-Nitro-m-cresol M. W. 153		2·0	·013	30	13·3		
		1·0	·0065	39	17·9		
		0·5	·0032	25	4·0		
		0·25	·0016	34	26·4		8·0
2-Nitro-m-cresol M. W. 153		2·0	·013	34	29·4		12·0
		1·0	·0065	29	17·25		
		0·5	·0032	25	4·0		
		0·25	·0016	19	10·5		
Trinitro-m-cresol M. W. 243		1·0	·0041	34	17·65		
		1·0	·0041	45	11·1		
		0·5	·002	28	14·3		
		0·25	·001	45	13·35		

Table V (*continued*).

[No. of eggs not hatching in controls taken as 20 %.]

Substance	Formula	Concentration		No. of eggs sprayed	% of eggs not hatching	% not hatching calc. on control	Remarks
		Gms. per 100 c.c.	Moles. per 100 c.c.				
3-Nitro-p-cresol M. W. 153		2·0	·013	44	54·5	43·1	
		1·0	·0065	40	12·5	—	
		0·5	·0032	60	16·65	—	
		0·25	·0016	14	35·7	19·6	
3 : 5-Dinitro-p-cresol M. W. 198		2·0	·01	28	96·3	95·3	
		1·0	·005	27	96·3	95·3	
		0·5	·0025	45	57·8	47·25	
		0·25	·00125	36	36·1	20·1	
o-Nitro-anisole M. W. 153		2·0	·013	27	25·9	7·4	
		1·0	·0065	40	10·0	—	
		0·5	·0032	53	9·4	—	
		0·25	·0016	62	9·5	—	
p-Nitro-anisole M. W. 153		2·0	·013	35	37·2	21·5	
		1·0	·0065	53	11·3	—	
		0·5	·0032	71	12·7	—	
		0·25	·0016	43	9·3	—	
2 : 4-Dinitro-anisole M. W. 198		2·0	·01	44	93·2	91·4	
		1·0	·005	47	53·2	41·4	
		0·5	·0025	25	40·0	25·0	
		0·25	·00125	50	35·7	19·6	

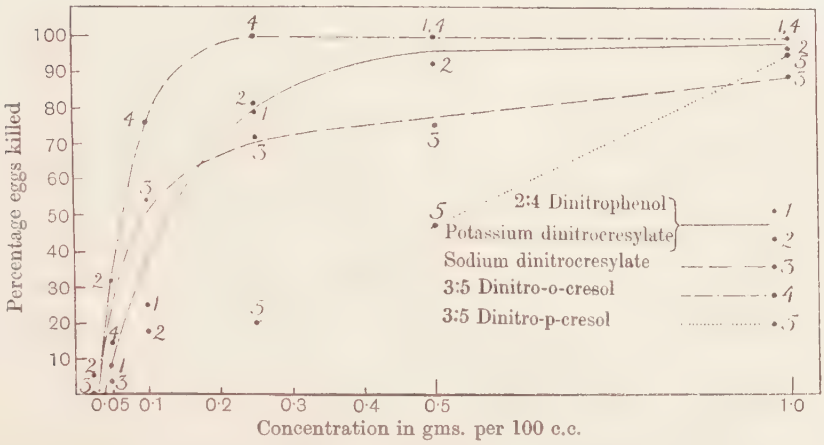


Diagram 11. Showing toxicities of dinitro-phenol and dinitro-cresols to eggs of *Selenia tetralunaria*.

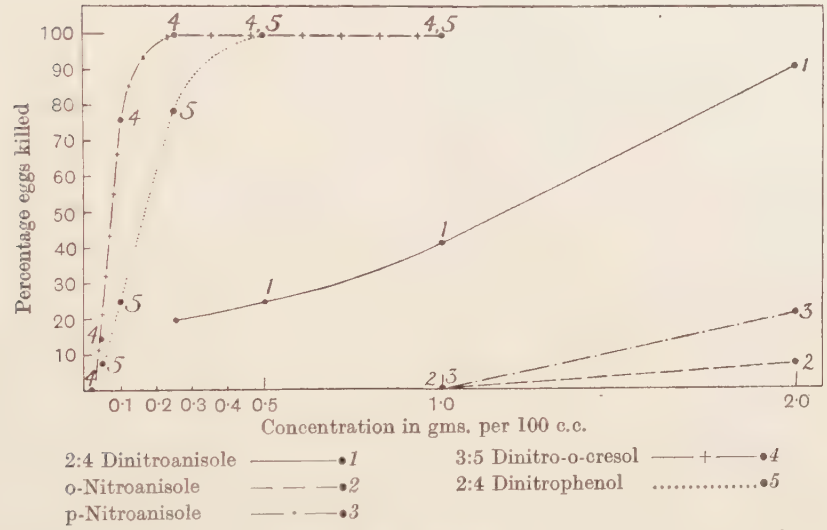


Diagram 12. Showing toxicities of nitro-derivatives of phenol, cresol and anisole to eggs of *Selenia tetralunaria*.

parallel sets lay within the lower and upper values of 2·3 and 11·3 respectively. The mean standard error of the average of a parallel series was 4·2, hence a difference of less than about 12 between the averages of such series would be insignificant. The average number of eggs failing to hatch being 12 per cent., it would be safe to adopt the value of 20 per cent. of eggs not hatching as the lowest indicating toxicity.

It will be seen from Table V that the relative toxicities of the chlor-, nitro- and chlor-nitro-benzenes run approximately in the same order as

that found in the tests with *A. rumicis*, though as was to be expected the actual toxicity to the eggs is in all cases less than to the aphides. Thus m-dinitro-benzene is more toxic than nitro-benzene, trichlor-benzene than the less chlorinated derivatives, and chlor-dinitro-benzene than the chlor-nitro-benzenes.

Nitrobenzene and the mono- and dichlor- and mono-chlor-nitro-derivatives of benzene are all without marked toxic action. The parallelism remarked on above is still more noticeable in the case of the phenols and cresols and their nitro-derivatives. There is no significant toxicity in the case of phenol and the three cresols at or below a concentration of 5 per cent. The introduction of one nitro-group into phenol leads to no marked increase in toxic properties. But again, as in the tests with *A. rumicis*, the introduction of a second nitro-group was coincident with a noteworthy rise in toxicity, 2 : 4-dinitro-phenol being highly effective at a concentration of 0·5 per cent. The substitution of a third nitro-group is correlated with a decrease in toxicity. Thus picric acid in saturated solution is without effect. The nitro-cresols offer a similar close parallelism, the introduction of one nitro-group having no material effect, while in the case of o-cresol the 3 : 5-dinitro-derivative shows the most pronounced poisonous properties.

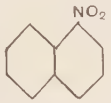
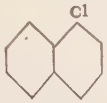
As will be seen, the results with 3 : 5-dinitro-o-cresol in this series of experiments are not in exact agreement with those recorded in the first series (Table IV). The differences are probably within the limits of error of the experimental method, but there is also a possibility that the explanation may lie in the fact that the eggs used in this series were from the summer brood of moths and were sprayed about 15 days before hatching whereas the eggs of the first series were the spring brood and sprayed 7 or 8 days before hatching. There may well have been significant differences in the resisting power of the two batches of eggs.

It is noteworthy that trinitro-m-cresol again shows no marked toxic action below 1·0 per cent. and that 3 : 5-dinitro-p-cresol is less toxic than 3 : 5-dinitro-o-cresol.

Contrary to the results of our experiments on aphides we were unable to find any very marked differences in toxicity to these eggs between the mono-nitro-cresol-derivatives.

An inspection of Table V will show that a similar set of results was obtained with the anisole derivatives. In this case however the parallelism with those obtained on *A. rumicis* is not complete. With the eggs, the 2 : 4-dinitro-derivative is distinctly more poisonous than the mono-nitro-derivatives, whereas the reverse was the case with aphides,

Table VI. *Toxicity of Naphthalene and derivatives to the eggs of* Selenia tetralunaria.

Substance	Formula	Concentration		No. of eggs sprayed	No. of eggs not hatching	% not hatching calc. on control	Remarks
		Gms per. 100 c.c.	Moles. per 100 c.c.				
Naphthalene M. W. 128							Not appreciably toxic at or below 10 %.
Tetra-hydro-naphthalene M. W. 132		10·0	·0758	21	95·5	94·4	
		5·0	·0379	29	20·7	—	
		2·5	·01895	29	10·35	—	
		1·0	·0076	39	2·56	—	
Deca-hydro-naphthalene M. W. 138							Not appreciably toxic at or below 10 %.
α-Nitro-naphthalene M. W. 173·1							Not appreciably toxic at or below 1 %.
α-Chlor-naphthalene M.W. 162·5		5·0	·0307	33	81·8	77·0	
		2·5	·01535	29	100·0	100·0	
		1·0	·00614	22	9·07	—	
		0·5	·00307	28	14·3	—	

a difference which we are unable to account for except by the assumption that the toxic action of dinitro-anisole to aphides is so slow as to be ineffective during the short time they were kept under observation or that the difficulties met with in spraying this compound were more successfully overcome in the tests with eggs than in those with aphides.

The use of dinitro-phenol and dinitro-o-cresol for spraying purposes necessitates the employment of an organic solvent. It was therefore of interest to test the sodium, potassium and barium salts, which are more soluble in water; and the results of these trials are also to be found in Table V. The concentrations in column 3 of this table are expressed in terms of the organic radical. Both the sodium and the potassium salts are somewhat less toxic, molecule for molecule, than either dinitro-phenol or 3 : 5-dinitro-o-cresol. The barium salt is distinctly less toxic.

In Table VI are set out the results obtained with naphthalene derivatives. The toxic effects are not marked. Tetra-hydro-naphthalene showed itself more poisonous than either naphthalene or deca-hydro-naphthalene. α-chlor-naphthalene was again the most toxic derivative of this series which was thoroughly tested; it requires however a fairly high concentration to be completely lethal.

Discussion.

It will be realised that many of the compounds discussed are interesting, in the first place, mainly from a theoretical point of view; but there are among them some which promise to be of practical interest also, as for example dinitro-o-cresol and its salts, dinitro-phenol, m-dinitro-benzene, chlor-dinitro-benzene, monochlor-naphthalene and one or two others.

The experiments recorded here were definitely designed to compare toxicities under standard conditions on two insects only (adult *Aphis rumicis* and eggs of *Selenia tetralunaria*) and direct deductions from the results as to the practical value of the substances tested are not justifiable. It was possible however to eliminate substances which are useless from this point of view and to select others for further investigation. In considering the practicability of a compound for use as an insecticide there are a number of factors besides toxicity which have to be taken into account—there are questions of price, ease of preparation and application in the form of spray, effect upon the plant, specificity towards different types of insects.

Among the compounds dealt with in this paper, dinitro-o-cresol and its salts stand out as specially highly toxic to both aphides and eggs. The sodium and potassium salts are readily soluble in water; and the latter mixed with soap was put on the market, as an insecticide, in the form of paste more than thirty years ago under the name of "Antinonnine" by a German firm. It was claimed that this material destroyed both biting and sucking insects.

So far as we have tested them, both dinitro-o-cresol and its salts are extremely injurious to foliage (hazel, hawthorn, bean) even at low dilutions; moreover *in the dry condition* the salts ignite very readily and are to some extent explosive. Little has been heard of the employment of "Antinonnine" in recent years and a search of the literature has revealed only one reference to a practical trial. E. G. Lodeman (3) in 1893 tried "Antinonnine" among other new insecticides and fungicides on apple, gooseberry, raspberry, blackberry and quince. He found that, at the rate of $\frac{1}{4}$ oz. to 1 gallon of water, damage to foliage, especially of apple and quince, was very severe and was still serious when half this strength was tried. Addition of an equal weight of lime reduced the degree of foliage injury. He states that the substance is dangerous to handle unless it is kept moist and concludes that "it possesses no practical value as a destroyer of sucking insects." This criticism does not however seem to preclude the use of dinitro-o-cresol itself as a winter wash on trees in a dormant condition. The great importance of a winter spray fluid which is definitely toxic to insect eggs has been brought into special prominence in recent years by the widespread use of the proprietary insecticide Carbolineum-Krimpen (4) and the numerous substitutes and imitations which are now on the market; and the marked toxicity of dinitro-o-cresol to insect eggs suggests that further work on this compound from the practical point of view is desirable. The result of a preliminary experiment indicates that dormant buds of apple are not damaged when it is used at a concentration of 0·25 per cent.; and trials on a practical scale are now in hand which should yield information as to the difficulties of preparation and application of a suitable solution or emulsion and as to the effect upon fruit trees and the toxicity to eggs or hibernating larvae under field conditions.

Of the other compounds mentioned above, m-dinitro-benzene and chlor-dinitro-benzene are also injurious to foliage. Their toxicity in our tests is appreciably less than that of dinitro-o-cresol but it is possible that they may be more readily and cheaply obtainable and are worth trying practically.

α-chlor-naphthalene, on the other hand, is not injurious to foliage of hazel, hawthorn and bean and its comparatively high toxicity to aphides and to the eggs of *Selenia* make it an interesting compound. As already mentioned there is a possibility that it may have powerful anaesthetic properties (like the chlorine-derivatives of benzene) which might cause the toxicity to appear greater than it actually is. This point needs further investigation before this substance can be considered to have a direct practical interest.

In the preceding sections we have given data concerning the toxicities to adult *A. rumicis* and the eggs of *S. tetralunaria* of a number of organic derivatives belonging to a limited number of chemical groups. There are references scattered through the literature about the toxicity of individual members of these groups; generally however these are meagre and are concerned with practical considerations only. A few papers have been published on the fundamental aspect of this problem in which however the toxicities are determined for the vapour phase of the compounds dealt with(5). Until further work has been carried out in order to ascertain how far deductions drawn from experiments carried out in one phase are applicable to another, the discussion of these results here would not seem to serve any useful purpose. Recently however Richardson and Smith(6) have tested the toxicity to *Aphis rumicis* of a considerable number of spray fluids containing organic compounds including a few which belong to the groups under discussion in this paper, and it is of interest to compare the results with those obtained by us. Their experimental method differed in several respects from ours. The aphides were reared on dwarf nasturtium plants (*Tropaeolum majus*) and were sprayed by means of a small hand atomiser, *in situ* on the plant without reference to the particular stages of the insect present. No solvent other than water was used; basic or acidic liquids were applied as salts and the solid compounds were either soluble in water or made so by conversion to a salt. 0·3 per cent. of fish oil soap was employed as an emulsifier for immiscible liquids and at this concentration was responsible for an average of 14 per cent. of deaths; but it was not considered necessary to deduct this figure from the toxicity values since the main object was a comparison of the different compounds. Results are expressed as the minimum toxic concentration in gms. per 100 c.c. (minimum capable of killing about 95 per cent.). The figures obtained by this method for compounds which we have dealt with in this paper are as follows:

	Minimum toxic conc. per cent.
Benzene	25·0
Toluene	16·0
Xylene	10·0
Phenol	5·5
Cresol (U.S.P.)	1·5
Chlor-benzene	9·0
Trichlor-benzene (comm.)	6·0

Comparison with the figures in our tables shows that we find the relative toxicities of benzene, toluene and xylene to be in the same order as found by these authors but in our experiments these substances proved less toxic, especially in the case of the first two. Phenol we find to be rather more toxic; the figure for cresol (U.S.P.), which is probably a mixture of the three isomers, is in good agreement with our figures for m-cresol and so far as the figures are comparable the results with monochlor-benzene also agree. We are at variance with regard to trichlor-benzene, but this difference cannot be stressed as our experience shows that the toxicities of the chlor-derivatives are not easy to determine on account of complications due to anaesthesis. Moreover trichlor-benzene having a high specific gravity is difficult to keep in suspension. It is also possible that our sample of trichlor-benzene contained some proportion of higher derivatives and may not therefore have been comparable with the sample used by Richardson and Smith.

Undoubtedly the most interesting compounds we have tested in this series are to be found among the phenols and cresols and their nitro-derivatives. Their importance arises from the wide variation in toxicity occurring between the isomers and the large changes in toxic action brought about by alterations in the number of nitro-groups in the molecule. We have found an interesting agreement with our results as to the relative toxicity to insects of phenol and its nitro-derivatives in a paper by Plantefol(7) on the toxicity of these compounds to a fungus, *Sterigmatocystis* (*Aspergillus*) *nigra*. Plantefol found that phenol and its nitro-derivatives were all toxic to *S. nigra*; that o-nitro-phenol was the least toxic of the mono-derivatives and p-nitro-phenol the most toxic; that 2:4-dinitro-phenol was about 100 times more toxic than phenol and about 10 times more toxic than the mono-nitro-phenols; and that 2:4:6-trinitro-phenol had only about the same toxicity as m-nitro-phenol. In every particular the relative toxicities of these compounds to our test-insects and to the fungus *S. nigra* run parallel, showing in both cases a maximum with the presence of two nitro-groups in the molecule.

There is also agreement between the relative toxicities of the three isomeric cresols to insects and their disinfecting values as determined by Ditthorn (8) who found the bactericidal powers to be in decreasing order: m- > p- > o-cresol. The differences between the three isomers both in toxicity to bacteria and to insects are, however, small though probably significant.

Looking at the results of our experiments as a whole, it does not seem possible to correlate toxicity in a satisfactory manner with any one simple chemical or physical property characteristic of the group of compounds. Moore and Graham (9) as a result of experiments on the toxicity of various compounds[1] to eggs of the potato beetle lay stress on the relation of boiling point and volatility to toxicity; whereas Richardson and Smith (*loc. cit.*) arrived at the conclusion, that "neither volatility nor boiling point nor chemical structure serves as a reliable index of the toxicity of organic compounds, although the latter is the best guide available at present." Such a conclusion deduced from experiments ranging over many compounds of widely differing structure and chemical and physical properties is rather to be expected. Although we have attempted in our survey of these groups to express our results in such a form as to indicate some of the fundamental facts concerned in insecticidal action, we do not attempt to synthesise the many scattered conceptions having a bearing on this subject into any simple generalisation regarding toxic action. Such a purpose could be achieved only by the intensive study of the toxicities of a number of closely related compounds using a large number of insects for the investigation of each, correlations being made with the change in physical and chemical properties arising from changing constitution. The nitro-derivatives of the phenols would appear eminently suitable for this purpose. If compounds of a different type are to be brought within the scheme the change in physiological action brought about by the alteration of the type would also require investigation. To the authors it does not appear possible at present to find some quite simple generalisation, whether physical or chemical, within the ambit of which can be included the complete explanation of the toxic action of many widely differing compounds. It would appear unquestionable that insecticidal action depends on a complex of physical and chemical properties which determine the physiological effects to which death is due. For example, solubility and the partition coefficients may determine the relative extent to which the compounds may be absorbed; the dissociation constants and other factors may decide the

[1] These investigators used their materials in an undiluted state.

ease with which penetration takes place; the type of radical may be responsible for the particular factor in the insect economy which shall be inhibited or stimulated; and the mutual effects of the various groups in the compound (effects which on modern views of alternate polarities depend in a simple way on the relative positions of the groups) may have a pronounced bearing on the reactivity of the compound. In the present stage of these investigations, it is not possible to analyse the bearing of such factors on our problem.

Barger and Dale(10) in their investigations upon the sympathomimetic action of amines summarise their conclusions in the phrase "On the whole then the least unsatisfactory view in the present state of our knowledge seems to us to be that which regards stimulant activity as dependent on the possession of some chemical property, the distribution and in the main the intensity of the activity as due to a physical property." With the substitution of "toxic" for "stimulant" and the realisation that more than one physical property may be involved, this conclusion adequately sums up our present views with respect to the fundamental nature of insecticidal action.

We are indebted to Professor G. T. Morgan for most kindly supplying us with samples of 3:5-nitro-2-chlor-toluene and 5:6-nitro-2-chlor-toluene; and to Mr G. P. Gibson for samples of 5-nitro-o-cresol and 6-nitro-, 2-nitro-, and 4-nitro-m-cresols.

Summary.

1. The toxicities of a number of chlor-, nitro- and hydroxyl-derivatives of aromatic hydrocarbons to *Aphis rumicis* L. (adults) and to *Selenia tetralunaria* Hufn. (eggs) have been determined.

2. The order of toxicity to the aphides of the hydrocarbons and their chlor- and nitro-derivatives is benzene < toluene < xylene < monochlor-benzene < p-dichlor-benzene < o-dichlor-benzene < trichlor-benzene < nitro-benzene < m-dinitro-benzene. The mono-chlor-nitro-benzenes have about the same toxicity as nitro-benzene; 1-chlor-2 : 4-dinitro-benzene is slightly less toxic than m-dinitro-benzene.

3. Phenol and the three isomeric cresols are toxic to aphides only at high concentrations. The mono-nitro-phenols and cresols are all more toxic than the parent substances, the order of toxicity of the phenols being o-nitro-phenol < m-nitro-phenol and p-nitro-phenol < 2:4-dinitro-phenol > trinitro-phenol; and the same order applies to the cresols and their corresponding derivatives.

4. α-chlor-naphthalene proved to be the most toxic of the naphthalene-derivatives tested.

5. With few exceptions, the relative toxicities of the various compounds to the insect eggs are approximately in the same order as to the aphides. The nitro-derivatives of phenol and the cresols were specially studied and it was shown that, as in the case of aphides, the dinitro-compounds are more toxic to eggs than either the mono- or the tri-nitro-compounds.

6. 3 : 5-dinitro-o-cresol is shown to have a toxicity both to adults of *Aphis rumicis* and to eggs of *Selenia tetralunaria* which is of the same order as that of nicotine.

7. Some of the compounds tested may prove to be of value as winter spray fluids for trees in a dormant condition although injurious to foliage, and experiments on a practical scale are in hand.

8. A consideration of the results as a whole leads to the conclusion that no simple generalisation as to the correlation of toxicity with any one chemical or physical property is possible in the present stage of our knowledge. It is probable that the nature of the toxic activity depends on chemical constitution whereas intensity of activity is determined by one or more physical properties.

LITERATURE CITED.

(1) Tattersfield, F., Gimingham, C. T. and Morris, H. M. (1925). *Ann. Appl. Biol.* xii, 61.

(2) Tattersfield, F. and Morris, H. M. (1924). *Bull. Ent. Res.* xiv, 223.

(3) Lodeman, E. G. New York (Cornell) Agric. Expt. Sta., Bul. 60 in Rpt. for 1893.

(4) Cf. Molz, E. (1911). *Centrlb. Bakt. etc.*, Abt. 2, xxx, 183, 204; van Poeteren, N. *Tijds. over Plantenzeiten*, 1912, xviii, 32: 1913, xix, 12; Schenk, P. J. (1924). *Floralia*, xlv, Jan. and Feb.

(5) Cf. Moore, W. (1917). *Jour. Agr. Res.* ix, 371: x, 365; Moore, W. and Graham (1918). *Jour. Agr. Res.* xiii, 523; Moore, W. and Graham (1918). *Jour. Econ. Ent.* xi, 70; Tattersfield, F. and Roberts, A. W. R. (1920). *Jour. Agric. Sci.* x, 199.

(6) Richardson, C. H. and Smith, C. R. (1923). *U.S. Dept. Agric.*, Bull. 1160.

(7) Plantefol, L. (1922). *Compt. rend.* clxxiv, 123.

(8) Ditthorn, F. (1918). *Centralb. Bakt. etc.*, Abt. 1, lxxxii, 483.

(9) Moore, W. and Graham (1918). *Jour. Agr. Res.* xii, 579.

(10) Barger, G. and Dale, H. H. (1910). *Jour. Physiol.* xli, 19.

A SYNOPSIS OF BRITISH *BIBIONIDAE* AND *SCATOPSIDAE* (DIPTERA)

By F. W. EDWARDS.

(*Published by permission of the Trustees of the British Museum.*)

(With 2 Text-figures.)

Although the larvae of most if not all the members of the small family Bibionidae are mainly saprophagous in their habits, several of them claim attention as secondary pests of some importance, attacking roots and tubers[1]. On the other hand, the activities of some of the species are undoubtedly beneficial, *Dilophus febrilis* being probably one of the most important agents in the fertilisation of fruit-blossom. The identification of the adults is therefore a matter of interest to economic entomologists, and as little has been published concerning the British species, the following keys are offered.

Several recent authors have separated the Scatopsidae from the Bibionidae as a distinct family. The differences are indeed so great and the two groups so well defined that this course seems to be quite justifiable from the standpoint of adult morphology. The chief distinctions are as follows:

Family Bibionidae. Eyes very large and bisected in the male, small and widely separated in the female, but with rounded outline in both sexes. Antennae inserted below the eyes, close to the oral margin. Labium and maxillary palpi well developed and protrusible owing to the extensive membrane between them and the head capsule; palpi with four segments. Thorax and abdomen usually conspicuously

[1] As has been pointed out by other writers, it is probable that in most cases the larvae are introduced in heavy dressings of farmyard manure. H. M. Morris (6) quotes a number of instances of damage by these larvae, and other cases have been mentioned in the *Review of Applied Entomology*. Mr E. R. Speyer informs me that he knows of a case where *Bibio johannis* caused serious damage to young larch trees, and Mr G. Fox-Wilson has kindly allowed me to mention the following cases of damage which have come to his notice: *Bibio hortulanus*, on *Anemone japonica* roots at Wisley, Feb. 1920. *Bibio marci*, celery and asparagus roots at Wisley and in other parts of S. England; complaints received annually, especially of damage to celery in trenches. *Dilophus febrilis*, hop roots in Surrey, 1921. *Scatopse* sp., said to have attacked potato tubers which were being forced in pots under glass.

hairy, though without macrochaetae. Male abdomen somewhat elongate, hypopygium of primitive and uniform type with ventrally-fused side-pieces (basistyles) and large simple claspers (dististyles). Legs rather long, the hind pair especially elongate. At least the four posterior tibiae provided with spurs. Pulvilli well developed, as large as the empodium. Subcostal vein present and fairly distinct, though not reaching the costa. Two basal cells, the upper one fairly large and reaching the middle of the wing. Veins *m-cu* and *Cu* 1 present.

Family Scatopsidae. Eyes moderately large and reniform in both sexes, more or less in contact above the antennae and also approximated below the antennae, which are well removed from the oral margin. Labium and maxillary palpi reduced, not protrusible, the palpi (in the British genera) with only one segment. Thorax and abdomen almost bare, the pubescence short and inconspicuous; the thorax often compressed, the abdomen short and stout. Male hypopygium complicated and very diverse in structure, but apparently never of the Bibionid type. Legs rather short. Tibiae without spurs. Pulvilli minute or absent, though the empodium is well developed. Subcostal vein absent. Only one basal cell, which is very small and at most about a quarter as long as the wing. Veins *m-cu* and *Cu* 1 absent (in the British genera).

Bibionidae.

The two British genera of this family differ as follows:

Pronotum large, with two transverse rows of short spines, one on the front and one on the hind margin. Front coxae almost or quite as large as the femora. Front tibiae with a ring of stout spines at the tip and with other spines about the middle. Costa extending well beyond the tip of *Rs*. *Cu* 1 not disconnected at base. *Dilophus*.

Pronotum smaller, without spines. Front coxae much smaller than the femora. Front tibiae with only two spur-like spines at the tip. Costa ending at or hardly beyond the tip of *Rs*. *Cu* 1 often disconnected at base. *Bibio*.

Genus Dilophus Mg.

We have four British species:

D. febrilis L. (*vulgaris* Mg.). Abundant everywhere in early spring, and apparently producing several broods during the year. Distinguished structurally from the other three species by the arrangement of the median spines on the front tibiae of both sexes, the spine towards the inner side being considerably nearer the base than the others. The body and legs are entirely black in both sexes; wings of male nearly hyaline, with a distinct black stigma; wings of female usually blackish, the tip lighter.

D. femoratus Mg. (*albipennis* Mg.) (Fig. 1 B). Almost as common as the last, but rather later in appearance. The spines in the middle of the front tibiae form a more or less regular transverse row. Body and legs of male all black; female black, the legs sometimes more or less reddish, especially the front coxae. Wings rather milky-white, with a distinct black stigma in the female, but practically none in the male; posterior veins all whitish. The male hypopygium is very deeply emarginate beneath.

D. humeralis Zett. (Fig. 1 A). Less common than the last, but widely distributed at least in the south of England: Denmark Hill, London; Southwold, Tangham Wood, and R. Deben, Suffolk (*Verrall*); Eastbourne, Sussex (*Nurse*); Felden, Herts.

(*Piffard*); Padstow, Cornwall (*Lamb*). Front tibiae as in *D. femoratus*, to which the male is superficially very similar, differing chiefly in the much shallower ventral emargination of the hypopygium and the rather differently shaped claspers; these latter however alter greatly in apparent shape in slightly different positions, as may be seen by comparing my figures with those of Lundström (5). The female can usually be distinguished by the red front coxae and sides of the pronotum, but specimens occur in which these parts are black; these may easily be mistaken for

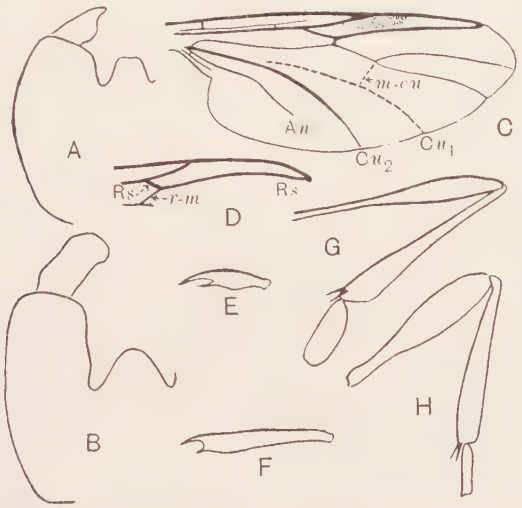


Fig. 1. A. Outline of hypopygium of *Dilophus humeralis* ♂, from beneath. ×40.
B. The same part in *D. albipennis*.
C. Wing of *Bibio hortulanus*, to show venation.
D. Part of wing of *B. anglicus*, showing distinction from *B. hortulanus*.
E. Front tibia of *B. reticulatus* ♂.
F Front tibia of *B. venosus* ♂, same scale.
G. Hind leg of *B. clavipes* ♂.
H. Hind leg of *B. anglicus* ♂.

D. femoratus, but in *D. humeralis* the posterior veins of the wing are always darkened and the membrane has a slight brownish tinge.

D. bispinosus Lundst. Concerning this species Mr J. E. Collin writes: "Two females are extensively tawny-yellow and have four strong spines on front tibiae arranged 2 : 1 : 1. These would appear to be *bispinosus* Lundst. (5) and not *ternatus*. Localities: Boyton, Suffolk, 27. viii. 07; Tangham Wood, Suffolk, 29. viii. 07. I do not know where Bloomfield's specimen (upon which *ternatus* was added to our list) is now to be found, but the probability is that it also is *bispinosus*." The arrangement of the front tibial armature and the yellowish thorax of the female make this species very distinct from the other three.

Genus Bibio Geoff.

The fourteen British species of this genus are all rather easily distinguishable, the only one which presents any difficulty being *B. varipes*. This is a variable species, especially as regards the amount and colour of the hair on the thorax of the male; the different forms have received separate names, but there appear to be no structural characters to separate them. Apart from the distinctions in colour, which are often extraordinarily great between the sexes of one species, the most remarkable differences between the species are to be found in the number of segments in the antennae, which seems to be constant for each species and is not influenced by sex; so far as I know no one has previously called attention to this variation. The male genitalia apparently offer no assistance in classification, being remarkably constant in structure throughout the genus. The species may be distinguished as follows:

1. Neither the front nor the hind tibiae swollen in either sex; outer spur of front tibia less than one-third as long as the tibia itself (Fig. 1 F). Antennal flagellum with 9 segments, the last very small. Body and legs black, with rather inconspicuous whitish hair; hind tarsi alike in the two sexes. Face of ♀ hairy. Wings nearly clear, with rounded black stigma; all the veins conspicuously darkened. Basal section of *Rs* about equal to *r-m*. *venosus* Mg.

   Front tibia in both sexes considerably swollen, also the hind tibia of the ♂; outer spur of front tibia over half as long as the tibia itself (Fig. 1 E). 2.

2. Front tibial spurs subequal in length. Antennal flagellum with 9 segments, the last very small. Face of ♀ bare. Body and legs black and black-haired. Hind tarsi alike in the two sexes. Wings of ♂ milk-white, with small indistinct stigma; of ♀ smoky black. Basal section of *Rs* about equal to *r-m*. *leucopterus* Mg.

   Outer spur of front tibia much longer and stronger than the inner. Antennal flagellum with not more than 8 segments. 3.

3. Basal section of *Rs* distinctly and often much longer than *r-m* (Fig. 1 C). Antennal flagellum with 8 segments, the last very small and indistinct. Face of ♀ conspicuously hairy. Wing-length 6–11 mm. 4.

   Basal section of *Rs* at most as long as *r-m* (Fig. 1 D); wing-length 4–7 mm. 6.

4. Femora red. Colour alike in the two sexes: body black, and usually also the tibiae and tarsi; wings nearly hyaline, with indistinct stigma, the anterior veins dark. First hind tarsal segment of ♂ rather swollen. *pomonae* F.

   Legs all black; tarsi alike in the two sexes, but colour of body different. 5.

5. Abdomen (♂, ♀) with black hair; *r-m* well marked.—Body all black. Wings of ♂ nearly hyaline, the costal cell blackish; in the ♀ almost entirely smoky, darker towards costa. *marci* L.

   Abdomen (♂, ♀) with whitish hair; *r-m* very short, almost obliterated.—Body of ♂ all black; wings nearly hyaline, costal cell darkened. Body of ♀ red, only the head, scutellum and thoracic pleura blackish; wings smoky, the tip generally whitish. *hortulanus* L.

6. Hind femora (♂, ♀) and tibiae (♂) slender almost as far as the middle, though outer half is swollen (Fig. 1 G).—Antennal flagellum with 9 segments. Eyes of ♀ smaller than usual. Legs of ♂ black; the outer half of the hind tibia and the first hind tarsal segment much swollen; legs of ♀ reddish, hind pair simple. 7.

Hind femora (♀) and tibiae (♂) commencing to swell much before the middle (Fig. 1 H). 8.

7. Tip of costal cell quite clear; body of ♀ mostly reddish, the mesonotum usually with three rather ill-defined blackish stripes. *clavipes* Mg.

Tip of costal cell blackish, the stigma extending over both sides of the tip of *R* 1; body of ♀ blackish. *lepidus* Lw.

8. Antennal flagellum (♂, ♀) with only 5 distinct segments.—Body black in both sexes; legs reddish, in the ♂ with the coxae and femora black, tibiae sometimes more or less darkened; first hind tarsal segment of ♂ slender. Wings of ♂ milky, with rather a distinct blackish stigma, posterior veins pale. Wings of ♀ slightly brownish, veins all dark, stigma black. Pubescence of ♂ black, of ♀ pale. *nigriventris* Hal. (♂ *lacteipennis* Zett.).

Antennal flagellum with 7 fairly distinct segments (8 in *ferruginatus*). 9.

9. Legs all black or blackish (♂, ♀). 10.

Legs bright red or ochreous (♀) or at least with reddish or brown hind tibiae and tarsi (♂); body all black, or at most the abdomen of ♀ brownish. 11.

10. Pubescence of abdomen black.—Body all black in the ♂, mesonotum and abdomen red in the ♀. Wings (♂, ♀) slightly smoky, the costal margin narrowly blackish. *anglicus* Verr.

Pubescence of abdomen whitish.—Thorax black (♂, ♀); abdomen all black (♂) or reddish with an indistinct dark mid-dorsal line (♀). Wings almost uniformly smoky, darker in ♀. *ferruginatus* L.

11. All veins almost equally dark.—Thorax and abdomen of ♂ with long dense pale hair. Femora of ♀ all blackened above at the tip. Halteres of ♀ black. Stigma well-marked, blackish. *reticulatus* Lw.

Posterior veins pale, or at least scarcely darker than the membrane. 12.

12. Stigma clear-cut, black.—Hair on ♂ thorax and abdomen all black, not very dense; first hind tarsal segment of ♂ about 3 × 1. Halteres of ♀ black; legs of ♀ ochreous. *johannis* L.

Stigma less conspicuous, light to dark brownish; hair on ♂ abdomen whitish, rather long and dense; legs of ♀ red. 13.

13. Wings brownish-tinged, stigma hardly darker (♂, ♀); first hind tarsal segment of ♂ about 3 × 1; halteres of ♀ black. *laniger* Mg.

Wings yellowish-tinged, stigma brown; first hind tarsal segment of ♂ about 4 × 1; halteres of ♀ light brown. 14.

14. Hair of ♂ thorax all black. *varipes* Mg.

Hair of ♂ thorax partly or all pale. *varipes* var. *hybridus* Hal.

Nearly all the above species are common and widely distributed, though *B. pomonae* occurs mainly in hilly or mountainous districts, *B. laniger* chiefly near the sea-coast, and *B. varipes* chiefly in woods, while *B. ferruginatus* is local. Most of the species are probably only single-brooded and have a rather short period of flight, the exception being *B. pomonae*, which apparently has a summer and an autumn brood. The majority are spring species, occurring in April and May; *B. hortulanus* flies in June, and *B. clavipes* and *B. lepidus* in September and October. *B. pomonae* and *B. hortulanus* fly singly, the males at least of most of the others being gregarious.

Scatopsidae.

This family has recently been revised by Enderlein (1), who has divided up the old genus *Scatopse* into a number of genera. While some of these appear to be well founded, others are based on slight and indefinite characters, and it seems better to treat them as groups or subgenera only. The key given below will separate the five British genera. The genus *Anarete* has been referred here by Schiner and more recently by Kieffer, but I emphatically share the opinion of Haliday and Enderlein that it is a Cecidomyiid allied to *Lestremia*.

According to Enderlein's interpretation the last of the faint veins on the wing of *Scatopse* is *Cu* 2, but I believe this view to be erroneous, and consider that in this group of genera *Cu* is simple, the last vein being really *An*. This seems to be the case in all the British forms, and also in the American genus *Forbesomyia*, but in the North European *Synneuron* and *Corynoscelis*, and the New Zealand *Canthyloscelis*, the arrangement of the veins in the cubital area (and in fact the whole venation) is quite different, and these should be treated as forming a separate subfamily. In the figure given of a *Bibio* wing, the veins *m-cu* and *Cu* 1 are shown dotted; if they were omitted the result would be a fairly close approximation to the venation of *Scatopse*.

1. Stem of median fork arising far beyond the bend of *Rs*, the *r-m* cross-vein therefore completely obliterated by fusion of *M* with *Rs* (Fig. 2 A); front or hind tibiae modified. 2.

   Stem of median fork arising at or just before the bend of *Rs*, *i.e.* no fusion of *M* with *Rs*, and *r-m* more or less indicated (Fig. 2 B–D); all tibiae simple; axillary vein absent or indistinct. 3.

2. Front tibia with the tip produced into a long spine; posterior tibiae with loose but rather distinct apical combs of spiny bristles; axillary vein absent. Aspistes.

   Front tibiae simple; hind tibiae with the tip considerably enlarged and flattened, and provided with a close comb of short fine bristles on the inner side (Fig. 2 K); axillary vein well developed and almost reaching the margin. Ectaetia.

3. Distinct though very fine macrotrichia on at least the apical portions of some of the thin veins, and also scattered over the lower half of the wing; upper branch of median fork more or less interrupted at the base. Psectrosciara.

   No macrotrichia on any of the thin veins nor on the membrane; upper branch of median fork not interrupted at base. 4.

4. Median fork very short and triangular, its base far beyond the end of the costa, which does not reach the middle of the wing. Swammerdamella.

   Median fork much longer, its base before the end of the costa, which reaches at least to the middle of the wing. Scatopse.

Genus Aspistes Mg.

The single European species (*A. berolinensis* Mg.) is apparently of rare occurrence in Britain, the only recent record being Porthcawl, Glamorgan (*Yerbury*). Life-history unknown.

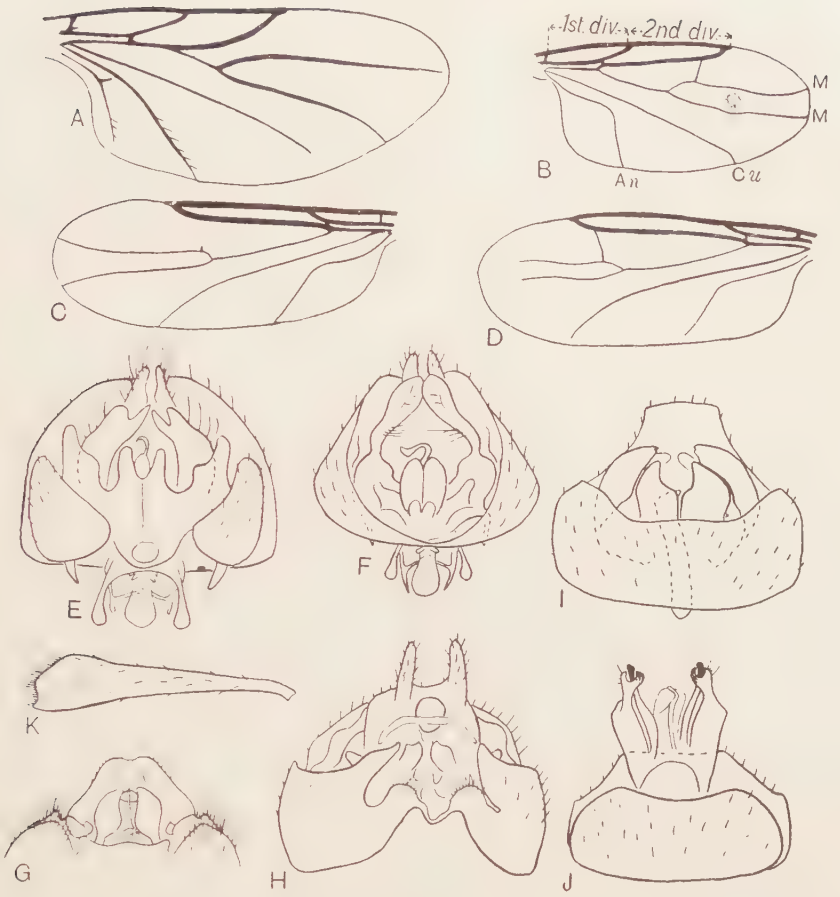


Fig. 2. A–D. Wings (× 20) of (A) *Ectaetia lignicola*, (B) *Scatopse bullata*, (C) *S. incompleta* ♂ and (D) *S. incompleta* ♀.

E–J. Hypopygium from beneath (× 55) of (E) *Ectaetia lignicola*, (F) *E. clavipes*, (G) *Psectrosciara palustris*, (H) *Ps. soluta*, (I) *Scatopse transversalis* and (J) *S. bullata*.

K. Hind tibia (× 40) of *Ectaetia lignicola*.

Genus ECTAETIA End.

Of this distinct genus there are three British species, none of them at all common. All that is known concerning their life-history is that *E. lignicola* was bred by Verrall from rotten wood.

1. Abdomen dull except at the base and tip; vein *An* with about 8–12 macrotrichia towards the tip; length 2·8–3·6 mm.; halteres black; last abdominal sternite of ♀ large and deeply emarginate. *platyscelis* Lw.

Abdomen uniformly shining; vein *An* with about 6–8 macrotrichia towards the tip; length 2·2–2·8 mm.; last abdominal sternite of ♀ smaller and less emarginate. 2.

2. Halteres black. *lignicola* sp.n.
   Halteres whitish. *clavipes* Lw.

E. lignicola sp.n. (Verrall MS.) (Fig. 2, A, E, K).

This rather closely resembles *E. clavipes* Lw., except that it is somewhat larger, of a distinctly more elongate form, and has black halteres. There are also quite well-marked differences in the male hypopygium, as shown in the figures.

Length of body or wing, about 2·8 mm.

Snailwell, Cambs., vi. 1906 (*G. H. Verrall*); bred from wood debris. 3 ♂, 3 ♀ in the British Museum, others in Mr Collin's collection.

Genus Psectrosciara Kieff.

In this genus I would include Enderlein's *Aldrovandiella* and *Anapausis*. All three groups possess distinct macrotrichia on the veins and membrane of the lower half of the wing, a point which was not noticed either by Kieffer or Enderlein, but which appears to me to form the best distinction from typical *Scatopse*. Apart from this, all the four British species have *M* 1 distinctly interrupted at the base, which is also the case in Enderlein's *Psectrosciara scatopsiformis*, though in the type species (*Ps. mahensis* Kieff.) the interruption is not complete, the vein being simply a little less distinct basally. *Anapausis* merges into *Psectrosciara*, but *Aldrovandiella* differs in several respects and may be recognised as a subgenus. Nothing has been recorded concerning the life-histories of any of the species.

1. *Rs* almost straight, and considerably shorter than the basal section of *R*; cell *R* 1 therefore small; *An* twice sharply bent; antennae with 12 segments; abdomen broad and depressed; halteres yellow (*Aldrovandiella*). 2.
   Rs curved, and much longer than the short basal section of *R*; cell *R* 1 therefore large; *An* with only one less conspicuous bend; antennae with 10 segments; abdomen more or less compressed (*Psectrosciara*). 3.
2. Second section of costa about one-third as long as the first; wings hyaline; front coxae clear reddish. *coxendix* Verr.
   Second section of costa about half as long as the first; wings milky; front coxae dark brownish. *halterata* Mg.
3. Thorax and abdomen shining black; halteres yellowish, the knob more or less darkened above; length 2–3 mm. 4.
   Abdomen dull black; halteres black; length 1·5–2 mm. *talpae* Verr.
4. Last abdominal tergite of ♂ swollen and ending in two long slender points which are directed downwards. *soluta* Lw.
   Last abdominal tergite of ♂ not swollen, slightly bilobed at tip, the lobes directed backwards. *palustris* sp.n.

Ps. coxendix and *Ps. halterata* are both widely spread in the South and East of England.

Ps. soluta (Lw.). This is the species which was recorded by Verrall as *S. inermis*, but all the British specimens I have seen appear to answer better to Loew's description of *S. soluta*; his name may be adopted, though he described the ♀ only. It is an abundant and widespread species in Britain. I have several times observed it swarming on wooden fence-posts in the hot mid-day sun, the swarm disappearing again in the afternoon.

Ps. palustris sp.n. (Fig. 2 G). Wings and coloration exactly as in *Ps. soluta*, but hypopygium of ♂ quite different (compare figures); also the segments of the ♂ antennae are relatively broader (segments 6–9 in *soluta* under, in *palustris* over twice as broad as long).

Chippenham, Cambs., 5. vi. 1906 and 3. viii. 1910, 3 ♂, 1 ♀ (*J. E. Collin*).

Ps. inermis (Ruthé). According to continental specimens in Mr Collin's collection this has the venation practically the same as in the two preceding, *An* being connected with the base of *Cu* in the same way. It differs chiefly in the conspicuously orange tip of the abdomen, brownish legs, and in having the microtrichia of the wing-membrane relatively coarse, so that the small macrotrichia are less conspicuous. The male hypopygium resembles that of *Ps. palustris*, but differs slightly. I have seen no British specimens.

Ps. talpae (Verr.). Reigate, Surrey; Barton Mills, Suffolk; Snailwell, Cambs. (*Verrall*).

Genus Swammerdamella End.

This genus includes a single European species, *S. brevicornis* Mg. Its characters are essentially those of *Scatopse*, but the very short costa and the short triangular median fork render the wings very distinct from those of all the other British species, and Enderlein's genus *Swammerdamella* may be accepted with little hesitation. The species is abundant and widely distributed in Britain and throughout Europe; it has also been found in Asia Minor and North America, Walker's *S. pusilla* being apparently the same species. Like several other species of this group, the flies frequent the flowers of umbellifers. The species is said by Müller (*Zeitschr. wiss. Insectenbiol.* xv, p. 120) to have been bred from the pupa of a *Phora*; the pupa may however have been dead when attacked.

Genus Scatopse Geoff.

Enderlein has proposed to restrict this name to the species of the *notata* group, which have a stump on vein *M* 1, erecting the genera *Reichertella* and *Rhegmoclema* for the species which have no stump, and *Holoplagia* for those with a complete transverse vein. But the stump, when present, is variable in length, being sometimes scarcely indicated, while in the female of *S. incompleta* and in occasional specimens of *S. notata* it forms a complete cross-vein. There seems therefore insufficient reason for separating *Holoplagia* and *Reichertella* from *Scatopse*. *Rhegmoclema* is rather better distinguished by the double bend in *An*, but here again the species of the *fuscipes* group seem to be more or less intermediate, and no very sharp distinction can be drawn. No British species of *Rhegmoclema* are known. Of *Scatopse* in the sense here adopted we have 16 species, most of which can be very easily recognised by the characters given in the following key.

1. A complete cross-vein connecting *Rs* with *M* 1 (Fig. 2 B); branches of median fork distinct and reaching the margin (*Holoplagia*). 2.

   This cross-vein absent or incomplete, or else (*incompleta* ♀) the branches of the median fork are abbreviated. 4.

2. Tarsi whitish; thorax brightly shining; wings rather milky; branches of median fork divergent apically. *albitarsis* Zett.
   Tarsi black; thorax scarcely shining; wings greyish; branches of median fork parallel. 3.
3. Last abdominal tergite of ♂ broad and slightly emarginate apically; wing of ♀ with a thickened white bulla in the median fork. *bullata* sp.n.
   Last abdominal tergite of ♂ produced into a large square-ended flap; wing of ♀ without bulla. *transversalis* Lw.
4. Costa reaching well beyond the middle of the wing; branches of median fork generally parallel or slightly and evenly divergent; *Cu* reaching the margin; *An* only slightly bent. 5.
   Costa hardly reaching beyond middle of wing, its second section distinctly shorter than the first; branches of median fork parallel basally, widely divergent apically; *Cu* not quite reaching the margin; *An* rather strongly bent. 14.
5. Vein *M* 1 with a stump of variable length, or at least with a definite though obtuse angle near the base. 6.
   Vein *M* 1 without any stump and even without any trace of an angle near the base; wings clear; halteres yellowish. 9.
6. Wings rather distinctly smoky, the posterior veins brownish; knob of halteres black. 7.
   Wings clear, the posterior veins colourless; halteres yellow or brownish. 8.
7. Legs all black.—Thorax shining black. Abdomen dull black. Antennae black. Wings of ♂ with a short stump near the base of *M* 1, branches of fork reaching the margin. Wings of ♀ with a complete though not very conspicuous cross-vein connecting *M* 1 and *Rs*; branches of fork not nearly reaching the margin. *incompleta* Verr.
   Tibiae broadly whitish at the base, and also pale at the tip, especially on the four anterior legs of the female, tarsi largely brownish. Thorax and abdomen dull black. Antennae black, but the last segment clothed with rather conspicuous white pubescence. Wings of ♂ with a more or less definite stump near base of *M* 1 (♀ apparently without this stump). *nigripennis* Mg.
8. Body shining black; second costal division much longer than the first; first hind tarsal segment of ♂ very short; length 2·5–4 mm. *notata* L.
   Body dull black; the two costal divisions about equal in length; first hind tarsal segment of ♂ longer than the second; length barely 2 mm. *tristis* Zett.
9. Body with brown or yellowish markings; at least the pleurae extensively yellowish and the posterior margin of the scutellum brownish; male hypopygium asymmetrical. 10.
   Body almost entirely black, more or less shining. 12.
10. Mesonotum and scape of antennae light to dark brown; femora and tibiae almost entirely yellowish; second costal division distinctly shorter than the first; stem of median fork angled in ♂, rather strongly curved in ♀, its base thickened; length 2·8–4 mm. *flavicollis* Mg.
    Mesonotum and scape of antennae black; femora and tibiae darkened apically; second costal division a little longer than the first; stem of median fork gently curved (♂, ♀); length 2–3 mm. 11.

11. Scutellum light or dark brownish; basal half of femora and nearly all the tibiae yellowish. *picea* Mg.
    Scutellum conspicuously margined with yellowish; femora nearly all black, tibiae with about the apical half black. *picea* var. *scutellata* Lw.
12. Length 1–1·5 mm.; legs all black. *pulicaria* Lw.
    Length 2–3 mm.; tibiae more or less distinctly lighter at the base. 13.
13. Tibiae more or less pale at the base only; male hypopygium with one rather stout twisted filament. *geniculata* Zett.
    Tibiae more extensively pale at the base, and also with an indistinct pale ring beyond the middle; male hypopygium with two long slender filaments. *bifilata* Hal.
14. Length 2–2·8 mm.; halteres black. 15.
    Length 1–1·3 mm.; thorax dull. 16.
15. Thorax dull; wings hyaline; body shorter and stouter. *fuscipes* Mg.
    Thorax shining; wings slightly milky; body more elongate. *nigra* Mg.
16. Tibiae broadly white at base; tarsi pale; halteres light brown. *minutissima* Verr.
    Tibiae nearly all black; tarsi black; halteres black. *litorea* sp.n.

S. albitarsis Zett. Widely distributed and rather common. Adults frequent flowers of umbellifers, especially in hot sun. Early stages unknown.

S. bullata sp.n. (Fig. 2 B, J). Body of a much broader and stouter build than in *S. albitarsis*; entirely dull black. Legs entirely blackish-brown. Antennae with 10 segments, of the usual length; the flagellar segments about twice as broad as long, except the last which is large and longer than broad. Thorax with short brownish pubescence, not at all compressed. Abdomen rather broad and depressed. Male genitalia as figured; the hypopygium more or less exserted, the last tergite not very large. Wings slightly greyish; anterior veins dark, posterior veins colourless; microtrichia normal, not denser at the tip in either sex. A well-marked cross-vein connecting *Rs* with *M* 1. Branches of median fork parallel in ♂, both distinctly curved upwards at the tip; in the ♀ the lower branch runs almost straight to the margin, and in this sex (not in the ♂) there is also a round, opaque whitish thickened spot near the base of the fork; this spot (bulla) is present on both wings of all the 9 females, and therefore cannot be accidental. *An* strongly curved, much as in the *fuscipes* group. Second costal division in both sexes slightly longer than the first. Halteres blackish.

Length of body or wing, about 1·8 mm.

Thetford, Norfolk, 17. vi. 1880, on tree trunk (*G. H. Verrall*). 1 ♂ (type), 3 ♀ in the British Museum, others in Mr Collin's collection. This species was recorded by Verrall, I think mistakenly, as *S. transversalis* Lw. It is probably myrmecophilous.

S. transversalis Lw. (Fig. 2 I). Differs from *S. bullata* chiefly in the male hypopygium (compare figures) and in the absence of the bulla on the wings of the female. Nearly all the known British specimens (as also Loew's original type) have been taken in association with the ant *Acanthomyops fuliginosus*; Mr H. Donisthorpe has also on one or two occasions taken it with *Formica fusca*. The species was identified by Verrall and recorded on several occasions by Donisthorpe as "*S. transversalis* var., *vel* n.sp.," but there can hardly be a doubt that it is the true *S. transversalis* of Loew.

S. incompleta Verr. (Fig. 2 C, D). Apart from the original locality (Abbey Wood, Kent) this species has only been found in the neighbourhood of Mildenhall, Suffolk. The sexual difference in venation is so remarkable that it seems worth while to figure it; the male has not hitherto been described.

S. nigripennis Mg. Apparently a rare species with us; the only recent captures I know of being two males from the New Forest (*Sharp*). There is apparently some sexual difference here also, the single female in the British Museum (the one recorded by Walker) lacking the stump on *M* 1 and having the tips of the tibiae distinctly paler than in the male. Probable synonyms are *S. infumata* Hal., *S. annulipes* v. Ros., and *S. fuscinervis* Lw., it also seems possible that the North American *S. tibialis* McAtee is the same species.

S. notata L. By far the commonest species of the genus, not only in Britain but in most parts of the world, it having been recorded from as far away as Iceland, Tasmania and New Zealand. The extreme shortness of the first hind tarsal segment of the male is a character found in no other species of the subfamily. The female tarsi however are normal. The early stages are passed in dung.

S. tristis Zett. only know this species from specimens in the British Museum so determined by Verrall, from Chippenham and Wicken, Cambs.

S. flavicollis Mg. A large species, very abundant in woods in the autumn, often occurring in great numbers on the leaves of trees.

S. picea Mg. Habits as in *S. flavicollis*, the two species often occurring together. The variety *scutellata* is at first sight quite distinct, but there are no obvious distinctions in the genitalia or other structural characters.

S. pulicaria Lw. Kelston, Cornwall (*Verrall*); no other recent records known to me. Walker's *S. integrata* is this species.

S. geniculata Zett. Widely distributed. Synonym: *S. consimilis* Hal.

S. bifilata Hal. Uncommon. Cusop, Hereford (*Verrall*).

S. fuscipes Mg. Widely distributed in Britain; occurs also (as a recently introduced species) in Tasmania and Peru; has been recorded from North America under the name *S. atrata*, though probably wrongly, since Say's description of *S. atrata* appears to indicate an *Ectaetia*. The larvae have been recorded as living in dung, while Dufour's record of *S. nigra* from rotten onions probably refers to this species. Synonyms: *S. recurva* Lw.; *S. simplex* Walk.; *Reichertella peruana* End. Also recorded by Walker under the name *S. minuta* Mg.

S. nigra Mg. I have only seen this from Denmark Hill, London (*Verrall*). Meigen's type, which I have examined, proves to be the same as Verrall's *S. subnitens*.

S. minutissima Verr. A sea-coast species, recorded by Verrall from Pagham, Sussex. I have taken a specimen on Dawlish Warren, S. Devon. The abdomen is over twice as long as broad in both sexes; male hypopygium exserted, the internal chitinisations (apodemes and vesicles) very large, more than half as long as the abdomen; last visible tergite of male broad at the base, suddenly narrowed on the apical half, tip truncate. Tibiae broadly whitish at the base; tarsi of the four posterior legs light ochreous except at the tips. Halteres and palpi pale brownish.

S. litorea sp.n. ♂. Dull black, like *S. minutissima*, and with an identical venation, but size even smaller, and differing conspicuously as follows: Palpi and halteres black. Legs almost completely black, only the tibiae narrowly and indistinctly paler at the base. Abdomen shorter and broader, barely 1·5 times as long as broad. Hypopygium small and hidden, quite different in the details of its structure; dorsally

there are three small hairy pieces, the lateral pair subquadrate, the median piece fingerlike, over twice as long as broad. Internal chitinisations much less than half as long as the abdomen, though much larger than the hypopygium. Last visible (seventh) tergite broadly triangular.

Length of body or wing, 1·0–1·3 mm.

Walton-on-Naze, Essex, 5. vi. 1908 (*G. H. Verrall*); type and one other ♂ in the British Museum, others in Mr Collin's collection. Humphrey Head, N. Lancs., 1–8. vii. 1923 (*F. W. E.*); 1 ♂ taken on sandy shore.

BIBLIOGRAPHY.

(1) Enderlein, G. (1912). Zur Kenntnis der Zygophthalmen. Über die Gruppierung der Sciariden und Scatopsiden. *Zool. Anz.* Bd. xl, 261–282.

(2) Loew, H. Fragmente zur Kenntnis der europäischen Arten einiger Dipterengattungen. [*Dilophus, Bibio, Scatopse,* etc.] *Linn. Ent.* i, 319–365.

(3) —— (1870). [On *S. transversalis, S. inermis,* etc.] *Zeitschr. Ges. Naturwiss.* xxxv, 1–6.

(4) Lundström, C. (1910). Beiträge zur Kenntnis der Dipteren Finlands. v. Bibionidae. *Acta Soc. Fauna Flora Fennica,* xxxiii, No. 1, pp. 1–15.

(5) —— (1913). Neue oder wenig bekannte paläarktische Bibioniden. *Ann. Mus. Nat. Hung.* x, 388–397.

(6) Morris, H. M. (1917). On the larval and pupal stages of *Bibio johannis* L. *Ann. Appl. Biol.* iv, 91–114.

(7) Verrall, G. H. (1886). [*S. subnitens, S. incompleta* and *S. minutissima.*] *Ent. Mo. Mag.* xxii, 180–181.

(8) —— (1912). [*S. coxendix* and *S. talpae.*] *Ent. Mo. Mag.* (n.s.), xxiii, 21–22.

(9) Walker, F. (1856). Insecta Britannica, Diptera, iii.

(*Received July 1st,* 1924.)

STUDIES ON *OSCINELLA FRIT*, LINN.

A PRELIMINARY INVESTIGATION OF THE EXTENT OF THE RECOVERY POWER OF OATS WHEN SUBJECT TO INJURY.

By NORMAN CUNLIFFE, M.A.

Christopher Welch Lecturer in Economic Zoology, University of Oxford.

CONTENTS.

I. Introductory Remarks.

The experiments of Roebuck and Brown (4) suggested that valuable data might be acquired from an investigation, similar in principle but suitably modified, of the extent of the recovery power of oats after injury such as is inflicted by the larvae of *O. frit*.

The problem of recovery after damage is difficult to elucidate as it would appear to depend on several factors, which may not be always constant in operation. This subject has been brought forward in a previous publication (2, p. 471), in which it was indicated that recovery "would seem to be dependent...mainly on the capability of the plant to produce effective tillers after the first shoots have been destroyed." By the term "effective tillers" was meant simply those shoots which would give normal yields, or approximations thereto, of grain and straw, within the *limited period of time* remaining after incurrence of the initial losses, during which growth and ripening could take place. Recovery should depend, therefore, on the rapidity of growth of the dormant buds after stimulation and on the capability of early ripening possessed by such shoots, factors which presumably vary with variety.

Another factor may be the method of entry of the larva into the stem; for example, when it bores directly into the stem, its line of drilling may include dormant or hidden buds. Other conditions being equal, the chances would appear to be even that such type of damage would be normally equally distributed in different varieties. But if, for example, one variety of oat elongated its internodes more rapidly than another, so that in one case the growing point was above the soil surface and in the other, below, when the stem was attacked, it would seem probable that in the former case direct entry might include the tiller bud or buds, while in the latter case these dormant tissues would escape[1]. When eggs are deposited below the soil surface, as apparently happens in some seasons, the intensity of damage would tend to be reversed.

Extent and degree of stem development at the period when a crop is subject to infestation is of extreme importance. All or none of the stems may be liable to attack, according to age, or on the other hand, considerable variations may occur within the one crop. For example, the first shoots may be immune from, and all subsequent shoots may be susceptible to, attack, as is often the case with a late sown crop or with a crop, the growth of which has been checked. Other possibilities readily suggest themselves, and indicate the necessity of investigating the inter-relationship of the stems with regard to production of straw and grain within a limited period of time (*vide* 3).

Environmental factors must necessarily greatly affect the recovery power of any one variety in any particular season, but may not greatly alter the relative degrees of recovery power exhibited by different varieties.

Although shoot destruction in spring may vary in extent from negligible damage to total loss of all shoots, being dependent on the size of the fly population and the age of the crop, intensity of damage to a shoot varies but slightly, because the larval drilling almost invariably includes the growing point of the shoot (*vide* 1, p. 69). The fact that a stem injured by a frit-fly larva rarely produces a panicle simplified the experimental procedure, inasmuch as the artificial destruction of the growing point of a stem would more or less faithfully imitate the vital part of the activity of the larva, which therefore could be imitated crudely on a small scale.

The following records refer to an investigation conducted during the season of 1924 and include two varieties of oat plants, to determine

[1] It was noticed that the first node of *Haig* was one inch above, while that of *Potato* was immediately below, ground level on May 27.

in the first instance what degree of recovery power, if any, an oat plant possessed, and in the second instance how the time factor operated with different varieties of oat. These records deal only with variations in yield under conditions constant per plot except for one factor and are not directly relative to the general problem of yield. Yield in the cereals has been discussed by Engledow and Wadham (3) and attention may be drawn to their valuable publications on this subject. As it was essential first to explore the unknown quality of recovery rather than to measure accurately its economic value, elaborate analyses of produce were not made and only a rough estimate of yield variations was attempted.

II. Method of Procedure.

This investigation was conducted in an outdoor insectary, constructed of frit-fly proof wire walls with a glass roof and having a floor area of 100 sq. ft. (natural soil). Fourteen plots of length $2\frac{1}{2}$ ft. and breadth $1\frac{1}{2}$ ft. were marked off in such a manner that accessibility to the plants was easy and room remained for cover plants where demanded. Six rows of seed were sown on April 13th in each plot, regularity in interval and depth being obtained by the utilisation of a sowing board. The spacing of the seed was two inches between the seeds in the rows and three inches between the rows and such that a seed in one row was planted midway between two seeds of the adjacent rows. Every plant was thus subject to similar conditions at the commencement of the experiment, while after treatment the damaged plants were liable to suppression to some extent, although to a much less extent than would occur in the field, owing to the wider spacing. Two varieties of spring oats were sown in alternate plots, namely *Sir Douglas Haig* and *Scotch Potato*, chosen because they are normally regarded as poor and prolific tillerers respectively. The seedlings were one inch high on April 26th and even in growth, although misses were numerous in spite of the selection of the seed[1].

May 27th was the date chosen for the first treatment, being about the time when a crop in the field would be liable to heaviest infestation (1), and the stems being in the late three leaf or very early four leaf stage. Several methods of endeavouring to kill the growing point of the stem were tried without success and the process finally adopted was simply

[1] The seed was supplied by Messrs Gartons, Ltd., Warrington, to whom my thanks are due. Before sowing, seed of doubtful quality was eliminated as far as possible by the floating method. The number of seed sown was 1162, from which about one thousand plants were expected to develop.

that of slitting the stem in the vicinity of the first node and removing the growing region with a fine needle and the assistance of a hand lens. Crude as it was, compared for example with cautery, the after-effects of this treatment closely resembled the results of fly attack, in that the central shoot died off in a few days, while the outer leaves remained capable of assimilation and respiration after the new shoots became vigorous. Very few, if any, of the lateral buds appeared to have been damaged, as the tillering of the treated plants was very regular and the stimulus evoked an immediate response.

By June 3rd these plants were tillering strongly. By June 6th these tillers averaged from two to three inches in length, extremes measuring one-quarter and four inches respectively, and on this date, the plants in the fifth drills were treated for the second time, two tillers being killed on each plant. By June 15th only ten plants in the whole series of the untreated plants had displayed any sign of tillering.

In simulating the action of the larva, six of the possible degrees of damage between the extremes, absence of damage and total loss, were selected for the purpose of experiment, one drill in each plot undergoing treatment according to the following scheme:

Drill 1. Control.

,, 2. Main stem and first two tillers undamaged.
Subsequent shoots killed on appearance.

,, 3. Main stem undamaged.
All subsequent shoots killed on appearance.

,, 4. Main stem growing point killed, May 27th.
All subsequent shoots undamaged.

,, 5. Main stem (May 27th) and first two tillers (June 6th) killed on dates indicated.
All subsequent shoots undamaged.

,, 6. Main stem undamaged.
First two shoots killed.
Subsequent shoots undamaged.

In the second drills, no plant produced more than three fruiting stems, therefore this drill in each plot was equivalent to the control drill[1].

The plants were harvested separately and analysed during the third week in August, when the main stems were ripe, *i.e.* when the major

[1] Owing to illness, the plants only received unskilled attention from the middle of June onwards; thus the treatment of drills 3 and 6 was very incomplete and the results therefrom nullified; also, the tillering details for the fifth drills were not recorded.

part of the crop would have been ready for harvesting under less severe treatment. The desirability of having a time limit precluded the alternative methods of allowing the crops to stand on the ground until every seed had ripened, or of collecting the ripe stems individually.

For each drill the following records were made:

(*a*) Number of plants per drill.

(*b*) Number of stems per plant, with and without panicles.

(*c*) Height of fruiting stem, from root to uppermost spikelet, to the nearest inch.

(*d*) Number of spikelets per stem.

(*e*) Weight of seed and straw, undried, to nearest decimal of a gramme and a gramme respectively.

In the case of the control drills, the data for the main stem and the tillers collectively were recorded separately. As the records have been subjected to statistical analysis, it is unnecessary to detail the actual observations and the analyses alone are presented in the next section.

III. Statistical Analysis of Observations.

In the first place it was necessary to examine the relationship between the number of plants per drill and total produce per plant, to determine whether variations in spacing (due to misses, etc.) were likely to affect the results seriously. This relationship being found to be linear, where individual measurements were available, as for example with the height of the fruiting stem, arithmetic mean values with their standard errors have been obtained by the frequency method. In the other cases, the arithmetic mean values derived from the relevant observations made on each plot were treated as a series for the measurement of divergence, as explained in a previous paper(2), with the aim of determining whether or no any observed differences were significant. To be significant, the observed difference (D) between two observations should exceed 2·57 times its standard error (E), which is the square root of the sum of the squares of the standard errors of the arithmetic means of the observations. The analyses of the records above mentioned are presented in the subjoined sections, the more interesting results being briefly indicated[1].

[1] Measurement of the standard errors of the differences by "Student's" method (*Jour. Agric. Sci.* IV, p. 131, 1911) indicated that the observed differences between the effects of the two treatments, stated here to be not significant for the most part, were in fact generally significant differences. For example, the difference in yield of grain with *Potato* becomes 0·51 ± 0·17 and with *Haig* 0·18 ± 0·27, these differences calculated by "Student's" method being significant. But as the study was rather in the nature of a preliminary exploration of positive recuperative powers, the use of the less precise method of analysis was of minor importance.

(*a*) *Mean number of plants per drill at harvesting.*

Stems treated	*Potato*	*Haig*
Control	10±0·7	9±0·6
Main	10±1·6	9±1·4
Main+2 tillers	8±1·2	7±1·4

In both varieties, checking the main stem alone failed to reduce the number of plants, while checking the first three stems produced by the plant induced an apparent loss of about 20 per cent. of the plants. These differences, however, were not significant, in neither case being more than 1·5 times their standard errors. In all cases the end plants of the drills were discarded as cover plants.

(*b*) *Mean number of fruiting stems per plant.*

Stems treated	*Potato*	D/E	% of control	*Haig*	D/E	% of control	% of Potato control
1. Control	1·70±0·08	—	100	1·34±0·08	—	100	79
2. Main	1·08±0·13	3·97	64	1·24±0·10	0·82	93	73
3. Main+2 tillers	0·92±0·18	3·97	54	0·95±0·09	3·25	71	56
Cf. 2 and 3		0·07			2·16		

A distinct difference between the varieties is brought out by these records. *Haig* produced fewer fruiting stems than *Potato* by 21 per cent. The loss of the main stem was more important with *Potato* than with *Haig*. *Potato*, after treatment, produced only 64 per cent. of the fruiting stems produced by the controls, while with *Haig* the observed difference was only 7 per cent. and not significant.

Considering now the more severe treatment, it will be observed that *Potato*, under these conditions, yielded approximately as many fruiting stems as before, but still of course many less than the controls. *Haig*, on the other hand, showed the effect of the more severe treatment, there being a significant drop of 29 per cent. in the panicle production compared with that of the controls. Under the second treatment, therefore, *Haig*, a poor tillerer, produced as many panicles per plant as did *Potato*, the more prolific tillerer. *Haig* exhibited excellent recovery power, as far as this characteristic was concerned, while *Potato* did not, the varietal difference being that the early formed stems of *Haig* matured more quickly than did those of *Potato*.

Considering shoot production (above 1 inch in length) per plant, irrespective of spikelet production, the following data were obtained at harvest time:

	Control	After loss of main stem	After loss of first three tillers
Potato	2·8±0·20	2·1±0·30	2·1±0·31
Haig	1·5	1·7	1·7

Contrary to expectation, the severely damaged plants did not produce seedless tillers in abundance, but concentrated on the maturation of seed on a few stems. *Potato* actually produced more stems per plant in the control drills than in the treated drills, the observed differences of 25 per cent. being significant and probably due to suppression. Even allowed a more extended growing period, it is doubtful whether seed production by the treated plants would have been markedly different, because of the weakness of the seedless tillers.

(*c*) *Height of fruiting stem to nearest inch.*

	Stems treated	*Potato*	% of control	*Haig*	% of control
1.	Control	43±0·6	100	38±0·7	100
1*a*.	,, (main)	48±0·7	112	39±0·7	102
1*b*.	,, (tillers)	37±1·0	86	34±1·4	89
2.	Main	42±1·1	98	36±1·0	95
3.	Main + 2 tillers	36±1·2	84	29±1·1	76

Of these data, all the observed differences over the value 2 were significant. Destruction of the main stem alone failed to lower the mean height of the fruiting stems in either variety. The additional loss of the first two tillers was, however, more severely felt, *Potato* losing 16 per cent. and *Haig* 24 per cent. in height.

Considering the main stems and tillers of the control plants separately (*vide* 1 *a* and 1 *b*), it will be noticed that the impression of height of crop would be due to the main stems, which were well above the mean of main plus tiller height in the case of the *Potato* but not in the case of *Haig*, due of course to the fact that *Potato* was the more prolific tillerer.

With *Potato*, even after the loss of the first three stems, subsequent stems attained the normal height of the tillers of the control plants: with *Haig*, however, recovery power was less pronounced, such stems as were produced under these conditions only reaching 85 per cent. of the normal tiller height.

(*d*) *Number of spikelets per fruiting stem.*

	Stems treated	*Potato*	% of control	*Haig*	% of control	% of Potato control
1.	Control	31±1·2	100	16±0·8	100	52
1*a*.	,, (main)	38±1·5	112	17±1·0	106	55
1*b*.	,, (tillers)	21±1·4	68	12±1·6	75	39
2.	Main	22±1·6	71	13±0·9	83	42
3.	Main + 2 tillers	15±0·9	48	8±0·7	50	26

Comparison of these data with those of the following sections indicates that height of fruiting stem alone would therefore be a poor criterion of recovery power. Variation in the available root area per shoot, acting contrary to the time factor, may partly account for these results.

Among these, all values above 4 were significantly different, both within each series and on comparison of the equivalent values of each series. Thus with *Potato* the observed differences within the series were significant, but with *Haig* this was not equally true.

Comparison of the two series shows that *Haig* only produced about half as many spikelets per panicle as *Potato*, under the same conditions. Even after losing the first three shoots the production of spikelets by *Potato* was equivalent to that of the control plants of *Haig*. The difference in spikelet production by the main stems and tillers of the control plants (1 *a* and 1 *b* respectively) was greater with *Potato* than with *Haig*, *Potato* tillers only carrying 55 per cent. of the number of spikelets borne by the main stems, the figure for *Haig* being 70 per cent. Thus the stems produced after loss of main stem only, were no more prolific than the normal tillers, at least in the case of *Potato*. The differences not being significant with *Haig*, rendered it impossible to judge from these experiments, whether such stems may not have been as capable of spikelet production as the controls. The loss of the first three shoots greatly affected the numbers of spikelets produced by subsequent shoots, a significant reduction of 50 per cent. being observed with each variety. In the case of *Potato* the spikelet production of these later stems was 29 per cent. below that of the normal tillers, as distinct from the main stems, the corresponding figure for *Haig* being 33 per cent. The growth period of the stems formed after the first treatment was approximately equal to that of the normal tillers, hence the equivalent spikelet production. The main stems were a month or more in advance of the secondary stems in all cases.

(e) *Spikelet production per unit drill.*

Knowing, per unit drill, (1) the number of plants, (2) the number of fruiting stems per plant and (3) the number of spikelets per fruiting stem, we may compare the total numbers of spikelets produced per unit drill under the three different treatments[1].

Stems treated	*Potato*	D/E	% of control	*Haig*	D/E	% of control
1. Control	527±48	—	100	195±20	—	100
2. Main	238±51	4·1	45	145±27	1·5	74
3. Main+2 tillers	111±28	7·5	21	53±12	6·0	27
Cf. 2 and 3		2·2			3·1	

[1] The error of the product of two quantities, $A \pm a$ and $B \pm b$ is

$$Ep = [(Ab)^2 + (Ba)^2]^{\frac{1}{2}}.$$

It would appear that *Haig* recovered from the less severe treatment much better than *Potato*, but that both varieties were unable to produce prolific tillers in a limited time after the loss of the first three stems. With *Potato* the experiment failed to distinguish between the effects of the two types of damage.

(*f*) *Yield of grain in grammes.*

A. *Per plant.*

Stems treated	*Potato*	D/E	% of control	*Haig*	D/E	% of control	% of Potato control
1. Control	2·43±0·18	—	100	1·66±0·27	—	100	—
2. Main	1·03±0·27	4·3	42	1·34±0·36	0·7	81	—
3. Main + 2 tillers	0·53±0·16	7·9	22	0·46±0·14	3·9	28	—
Cf. 2 and 3		1·6			2·3		
1*a*. Control (main)	2·09±0·12	—	—	1·45±0·12	—	—	—
1*b*. „ (tillers)	0·58±0·05	—	—	0·21±0·07	—	—	—

B. *Per unit drill.*

1. Control	24·3±2·44	—	100	14·95±2·65	—	100	62
2. Main	10·3±3·16	3·5	42	12·05±3·78	0·6	81	50
3. Main + 2 tillers	4·2±1·44	7·1	17	3·22±1·14	4·1	21	13
Cf. 2 and 3		1·8			2·2		

The variations in yield of grain were closely correlated with the variations in spikelet production per unit drill, as one would expect. The time limit seriously affected the weight of grain produced by the stems of *Potato* formed after the loss of the main stem, reducing the yield by 58 per cent. *Haig* made a good recovery, the yield being only 19 per cent. less than that of the control plants and this difference was not significant.

The yield of grain by the tillers formed after the second treatment was significantly less than that of the control plants but, low as it was, not significantly different in these experiments from the yields obtained from the plants which received the first treatment only.

Data for the yields of grain and straw from the main stems only and from the tillers collectively have been included in this and the following table. For the purpose of arriving at these arithmetic means, observations from all the drills equivalent to the controls were utilised and therefore they were not exactly comparable with the data given for the control plants, though their relative values should be more accurate. With *Potato*, 78 per cent. of the grain yield and 65 per cent. of the straw yield of the undamaged plants was contributed by the main stems only, the corresponding figures for *Haig* being 87 per cent. and 78 per cent. respectively. These figures indicate the extreme importance

of urging early growth with late sown crops, especially in the case of a variety such as *Haig*.

(*g*) *Yield of straw in grammes.*

(A) *Per plant.*

Stems treated	*Potato*	D/E	% of control	*Haig*	D/E	% of control	% of Potato control
1. Control	12·0±0·99	—	100	5·6±0·73	—	100	—
2. Main	6·2±0·92	4·3	52	4·0±0·80	1·6	71	—
3. Main+2 tillers	4·0±0·66	6·8	33	2·4±0·27	3·2	43	—
Cf. 2 and 3		2·0			1·6		
1*a*. Control (main)	8·5±0·44	—	—	3·9±0·31	—	—	—
1*b*. „ (tillers)	4·5±0·67	—	—	1·1±0·32	—	—	—

(B) *Per unit drill.*

1. Control	120·0±12·8	—	100	50·4±7·3	—	100	42
2. Main	62·0±13·5	3·1	52	36·0±9·1	1·2	71	30
3. Main+2 tillers	32·0±7·2	6·0	27	16·8±3·8	4·1	33	14
Cf. 2 and 3		2·0			1·9		

The remarks made concerning yield of grain apply equally to the yield of straw, except that here the losses, though of the same order, were not quite so heavy.

IV. Summary.

In the summer of 1924 an investigation of the extent of the recovery power of oats after injury approximately similar to that caused by *Oscinella frit* Linn. was conducted under experimental conditions. The larval attack in spring was simulated by killing the growing points of the stems [(1) main stem only and (2) main stem plus first two tillers] and growth records were obtained after a *limited period of time*. The varieties of spring oat, *Scotch Potato* and *Sir Douglas Haig* were used, being prolific and poor tillerers respectively. The oats were sown on April 13th, were above ground on April 26th, treated (1) May 27th and (2) June 6th, the crop being harvested during the third week in August.

1. Undamaged *Haig* plants produced a less number of panicles than undamaged *Potato* plants by 21 per cent. With *Potato*, loss of main stem alone and loss of the first three shoots caused a reduction of about 40 per cent. in panicle production. With *Haig*, recovery after subjection to the first type of damage was almost complete, and as good as that exhibited by *Potato* after the second type of damage.

2. With regard to height of fruiting stem, a reduction of about 20 per cent. was observed after the more severe treatment with both varieties.

3. Spikelet production varied greatly with variety, *Potato* carrying twice as many spikelets per fruiting stem as *Haig*. Tillers produced after the loss of the main stem carried no more spikelets than the normal tillers, *i.e.* about 30 per cent. less than the mean of the stems of the control plants. Tillers formed after the loss of three stems exhibited a reduction of 50 per cent. in spikelet production.

4. Yield of grain was not recovered by *Potato* after loss of the main stem (reduction 60 per cent. by weight), whereas *Haig* to a great extent recovered its yield. Loss of the first three stems caused a reduction of 80 per cent. in both cases, the additional reductions not however being significant in these experiments. Owing to the production of a greater number of seed, however, *Potato* yielded almost as well after losing its main stem as *Haig* did when undamaged. The variations in yield of straw were of the same order as those observed for yield of grain.

5. Recovery power after injury would appear to be much more marked with *Haig* than with *Potato*, and cannot necessarily be forecasted from a knowledge of tillering capacity. Under field conditions, where capability of covering the ground after losses may be comparatively small, as in the case of a weak tillering variety (although poor initial tillering capacity may be compensated for by an increased population), an oat like *Potato* might give a better yield of produce than one like *Haig*, even although *Haig* may exhibit greater recuperative powers than *Potato*. This problem is of course intimately related to the general problem of yield, which itself still awaits solution.

REFERENCES.

(1) Cunliffe, N. (1924). Further Observations on the Prevalence and Habits of *Oscinella frit* Linn. *Ann. App. Biol.* xi, 54–72.

(2) Cunliffe, N. and Fryer, J. C. F. (1924). *Oscinella frit* Linn. An investigation to determine how far varietal differences may influence infestation of the Oat Plant. *Ibid.* xi, 465–481.

(3) Engledow, F. L. and Wadham, S. M. (1923). Investigations on yield in the Cereals. I. *Journ. Agric. Sci.* xiii, *et seq.*

(4) Roebuck, A. and Brown, P. S. (1923). Correlation between Loss of Leaf and Damage to Crop in late attacks in Wheat. *Ann. App. Biol.* x, 326–334.

(*Received Nov. 20th*, 1924.)

A DISCUSSION ON THE GENERAL PRINCIPLES THAT SHOULD UNDERLIE GOVERNMENT ACTION RESPECTING FUNGICIDES AND INSECTICIDES

ORDINARY MEETING. *NOVEMBER* 14, 1924.

OPENING ADDRESS BY MR J. C. F. FRYER.

In approaching the subject—the Principles that should underlie Government Action respecting fungicides and insecticides—it seems desirable to restrict somewhat the meaning which should be given to the term "action." In theory "Government Action" might comprise any form of intervention, passing from fundamental research on the one hand to the manufacture and sale of insecticides and fungicides on the other—or even to undertaking the spraying of orchards on behalf of their occupiers.

The manufacture or sale of insecticides and fungicides by the Government, however, is obviously outside practical politics and is doubtless not intended as a subject for this discussion, while investigation is governed by the policy already laid down as to Government assistance in regard to agricultural or horticultural research which already includes work upon insecticides and fungicides. The subject for discussion, as restricted, then is somewhat as follows:—Granting no change in the policy as regards agricultural and horticultural research or in the existing conditions as to the supply and use of insecticides or fungicides, what principles should govern official intervention in regard to these articles?

The dominant principle in this matter is of course obvious and is that the Government should give every assistance within its means to farmers and fruit-growers in controlling pests.

Within the limits just mentioned there would seem to be two directions in which a Government might attempt to assist growers in regard to insecticides and fungicides. In the first place it might undertake to test articles of this nature which are already on the market and to publish reports on these tests for the public benefit. In the second place it might proceed to legislation in regard to the sales of insecticides and fungicides.

Each of these alternatives must be considered separately, but before doing so it is necessary to point out that there are two perfectly distinct classes of insecticide or fungicide on the English market. There is first the substance of which the active chemical ingredient is declared—as, for instance, lead arsenate paste. In such a case there may be present more or less of substances which act as diluents, such as water in lead arsenate paste, but the toxic ingredients are known to the purchaser.

In the second class of insecticide or fungicide, the composition of the substance is kept secret from the purchaser, who buys it under some fancy name such as "Killbug" or "Capsicide" with little or no information as to what the article contains. For the sake of brevity the first class or compound we may call the "Declared" class, while the second may be called the "Secret" class.

It is important to realise that while the Declared compounds can only vary in the amount of the toxic chemical or of any diluents present, the Secret class may be changed at the will of the manufacturer, both quantitatively and qualitatively, also that while the former class may be made by several firms the latter is the property of but one only.

Now reverting to the two directions for Government intervention and examining first the possibility of testing insecticides and fungicides, the position so far as the Declared class is concerned is relatively simple. The existing research and advisory organisation can test any given chemical both in the laboratory and under commercial conditions in the field, the results can be published, and the Government directly or indirectly can ensure that the practical deductions are available and are demonstrated to growers. As a matter of fact, practically every chemical of the Declared class which is now on the market has been dealt with in this manner and apart from the development and perfection of the machinery already available, no change appears needed to meet the needs of the case.

As regards the Secret class, however, the position is quite different. Here it is not a case of testing a definite chemical—which tests will apply to that chemical by whomsoever it is sold. It is instead the testing of a mixture, the composition of which is more or less unknown and which is the property of some one individual or firm. Of course, theoretically, the same tests could be applied to the Secret article as can be done in the case of the Declared, but in practice we are at once confronted with two difficulties. In the first place the proprietor of the compound may find it to his advantage to change his mixture and even during one season two different batches not exactly the same may be on the market. An official test would thus apply only to the batch from which the sample received was actually drawn, and where—as in most cases—a test must extend over more than one year, the maker might well claim when the report was issued that the compound he was selling, though sold under the same name, was vastly superior to that which had been tested. If the report was adverse the temptation to make such a claim would be very strong, and indeed if an improvement had in fact been made, the claim might be substantiated. It might be suggested that this difficulty would be overcome if a guarantee was obtained from the maker that he would not vary his product during the period of the test, but then what maker would bind himself to make no change or improvement for perhaps two or three years? It would be unreasonable to expect him to do so.

In the official testing of "Secret" insecticides there is thus one real difficulty in making the trials of any practical use. It is not, however, the only difficulty. Whereas in a test of—say—diplumbic lead arsenate, the results apply to this chemical by whomsoever it is sold, in the case of the secret class the tests would only apply to one particular maker. There is nothing to prevent other makers from marketing practically the same mixture under another name, and then each brand has to be tested. Further, since Government is involved, the advantages or disadvantages of having their products tested would in fairness have to apply to all firms concerned in the trade, and there would thus be an enormous number of tests to be carried out, involving facilities and staff far beyond anything contemplated at present, and indeed, in my own opinion, greater than the practical results would warrant in the absence of any measure to secure constancy in the products tested.

My own conclusions are, therefore, that so far as official and public testing are concerned, such tests can prove very valuable in regard to the Declared insecticide or fungicide, but that they are not feasible in the case of the Secret preparation unless reinforced by some legislative measure.

We are thus brought to the second possible line of Government action—the passing of legislation to control the sale of insecticides and fungicides, and here again it is necessary to consider separately the Declared class and the Secret class. So far as the Declared class is concerned, it might at first sight be thought that legislation would not be required and that the necessary safeguards for the purchaser would already be provided for by the Acts governing the sale of merchandise in general. This, however, is not quite the case, as may be seen by reference to such a substance as lead arsenate paste. Of six well-known brands which were analysed, the content of arsenic oxide proved to be as follows: 12·99, 15·62, 21·76, 17·04, 15·93, 20·38. Now since the toxicity of lead arsenate depends—other things being equal—on the percentage of arsenic oxide it contains, it is quite evident that if 1 lb. of paste to 25 gallons of water is the correct strength for a paste containing 20 per cent. arsenic oxide, it will be too low for another containing 12 per cent. arsenic oxide. It is not argued that a paste containing 20 per cent. As_2O_5 is necessarily better than one with 12 per cent., or that a firm selling the latter is doing anything discreditable. It might even be that a 12 per cent. paste, owing to its physical condition, would at dilutions giving an equal As_2O_5 content, produce better results than a 20 per cent. paste. The point is, however, that the grower should have the facts before him, for otherwise he will treat all lead arsenate pastes as of equal strength.

Then again, insecticides or fungicides may contain matter which is harmful to the plants treated. Reverting to lead arsenate as an example, it may be mentioned that four of the samples tested contained practically no water-soluble arsenic but two contained measurable quantities, which in one case was practically 1 per cent. Now 1 per cent. water-soluble arsenic may lead to serious foliage injury, and although no doubt the grower could claim for any damages which resulted, he would in many cases have the greatest difficulty in proving his claim, and it would obviously be better to avoid such litigation by preventing the sale of arsenate pastes containing more than a harmless quantity of water-soluble arsenic. Much the same reasoning applies to other insecticides and fungicides in the Declared class, and it would seem that the grower would benefit by legislation which entailed first a declaration of the content of the active ingredient in substances of this class, and secondly the restriction of the content of injurious chemicals to such proportions as would prove harmless in practice.

The Declared class of compound would thus prove fairly simple to deal with, but again the Secret class provides very serious difficulties. The mere fact that these preparations are secret renders impossible any mild form of regulation and there would seem to be but two possible directions in which legislation could proceed. In the first place it might be provided that such insecticides or fungicides should be subject to some official test, which might either result in the granting or withholding of licences prior to sale, or in prosecution should the articles, when on the market, be found not to fulfil their makers' claims. The licensing of secret articles would, from the technical standpoint, be so difficult to administer and so irritating

and troublesome to the licensees that it is not likely to commend itself to many. The penalising of articles for which false claims were made cannot however be dismissed so easily. In principle, there appears to be every reason why such articles should be penalised, but without a very perfect organisation, the difficulties of administering the necessary legislation seem rather appalling. One can imagine the legal arguments as to whether because Article A killed two wireworms per thousand, its claim to destroy these pests was substantiated, or again whether because Article B failed to kill ten caterpillars per cent., its sale as a caterpillar destroyer was prevented. The prospect can hardly be inviting to anyone likely to be implicated in the matter, but nevertheless legislation of this type cannot be altogether overlooked, as it is in being in another country—as will be shown subsequently.

The only remaining scheme of legislation would seem to be the bold one of compelling the sellers of secret compounds to declare the names and percentages of the active ingredients in their articles, which is tantamount to doing away with the Secret class of product altogether. From the growers' standpoint there would seem to be obvious advantages in this, but it has been stated that the administration of this type of Act would give rise to serious difficulties on the chemical side to which it is hoped other speakers qualified in chemistry will refer, while from the point of view of the manufacturer, much of the incentive to discover and introduce more efficient chemicals would disappear, with ultimate loss to the grower. The Secret insecticide and fungicide therefore form a stumbling-block in the consideration of legislation as they did in regard to official trials, for apart from any question of the practical politics of introducing measures to deal with them, it is not easy to reach a conclusion as to the merits of any scheme. Naturally, under these circumstances, the question to ask is what are other countries doing in the matter?—a question which has proved far more difficult to answer than might be expected. However, so far as the information goes, there appear to be three types of legislation in being. The first, and I believe the oldest, may be described as of the public health type, and was solely devised to prevent genuine or supposed dangers to the public health which might result from the spraying of plants with poisonous substances. This type of legislation need not detain us, for if it was proved that there was a real and definite danger, the need for Government action would be obvious.

The second type of legislation may be termed the "indirect," for it is not aimed primarily at insecticides or fungicides. It usually consists in a law to compel fruit-growers or farmers to control certain pests—as, for instance, by spraying plants or dipping animals with an officially approved insecticide or fungicide, and the necessity for the use of an approved chemical gives the Government concerned a virtual control of certain insecticides or fungicides. This type of legislation is, I believe, operative in this country in connection with Sheep Scab, and in some countries in regard to orchard pests such as the Codling Moth, but it clearly does not meet the general case under consideration.

Of the third type of legislation—that which is aimed directly at insecticides and fungicides, I have only been able to get details of the law in the U.S.A., which is in some ways rather complicated and difficult to follow, since there is both a Federal Act and also in many States a State Act as well. The Federal Act has five main provisions which are roughly as follows:

The first states the minimum percentages of arsenious or arsenic oxides which

must be contained in Paris green and lead arsenate, also the maximum content of water and water-soluble arsenic.

The second provides that no insecticide or fungicide must fall in strength or purity below the professed standard under which it is sold, nor must it contain any ingredients injurious to vegetation if advertised for the treatment of plants.

The third states that any insecticide or fungicide is misbranded if the label or literature concerning it comprises a false, deceptive, or misleading statement.

The fourth is that insecticides and fungicides other than Paris green or lead arsenate which contain arsenic in any form must bear a statement showing the percentage of such arsenic and the percentage of arsenic in water-soluble form.

The fifth provision is that insecticides and fungicides other than Paris green and lead arsenate which contain inert substances must either bear a statement giving the percentage of each inert substance, or alternatively a statement giving the percentage of each active ingredient and the total percentage of inert substances.

This Act, therefore, is decidedly drastic, for in the first place the provision as to misbranding is a powerful weapon for use against the advertiser of misleading statements, while the fifth provision, compelling the declaration either of all active or all inert ingredients must obviously hamper very considerably the sale of the secret class of article. It is thus a measure which, while allowing for the existence of secret insecticides or fungicides, does nevertheless attempt a very considerable control of their sale.

The State laws, so far as they have been examined, appear very largely to duplicate the Federal laws, but to arrange for State as opposed to Federal administration.

On general principles the U.S. law would appear to be sound for it is clearly not desirable that growers be deceived into purchasing substances which are useless, or relatively useless, for the purpose for which they are advertised. As to how these laws work in practice I have no first-hand information, nor do I know whether—and if so, how—the administrative difficulties which appear inseparable from this type of legislation have been overcome. From the number of reports of cases taken by the Insecticides and Fungicides Board, it is quite clear that a large number of fraudulent articles have been detected and prevented from further sale, while I am informed by the Chairman of the Board that the vast majority of contraventions are not brought into court at all owing to the willingness of the offenders to put matters right on being warned. As to whether the principles of the U.S. law could now or ultimately be extended to British conditions, I shall not venture an opinion, for the decisive factors are by no means only technical in character, and a much closer study of the American results would be needed to justify any view.

In conclusion, and to sum up the position as it appeals to me at present, perhaps I may be allowed to refer to Leaflet No. 363 of the Ministry of Agriculture. In it you will see that in 1920 at the request of the Chamber of Horticulture, and in co-operation with one of its Committees, a bill was in fact drafted to deal with the Declared class of insecticide and fungicide. This Committee contained representatives not only of the horticultural industry but also of many of the foremost firms of insecticide and fungicide manufacturers. For reasons of economy the Bill was not proceeded with but it may be deduced that both the makers and the users of the articles concerned were at one in regard to the desirability for legislation for the declared class of insecticide and fungicide. No arguments have been advanced

against Government action in regard to this class of compound and such intervention may be claimed as sound in principle. It is possible that Government intervention should be extended in principle to the Secret type of article but until definite proof is forthcoming it may be permissible to suggest that an absence of Government action with regard to the Secret class of insecticide or fungicide has a logical basis, since it is reasonable to claim that if the grower has at his command Declared insecticides and fungicides properly tested and guaranteed, he should take his own risk if he prefers to use those in the Secret class. Such a policy would appear good at the moment, but it should not be forgotten that it only remains so as long as the Secret class does not greatly exceed in efficiency the Declared class. If Secret insecticides and fungicides should definitely establish their ascendancy in the farming and fruit-growing world, Government action with regard to the other class would be of little public use and a call for the testing or regulation of Secret compounds would probably be made.

On re-reading these notes I feel that little attention has been given to the subject announced for the meeting—that is to say, the Principles underlying Government action—but this seems to me inevitable, for to my mind there is only one big principle involved—that the Government should do everything within its means to assist in the production of food.

Mr Lobjoit said: The title of the discussion assumes that Government action is necessary in connection with Insecticides and Fungicides. This, however, is not taken for granted in all quarters. A high authority has dubbed the Insecticides and Fungicides Bill as "grandmotherly" legislation. A case for action must, therefore, be made out.

The maxim "caveat emptor" has not been deemed a sufficient answer in regard to the purchase of seeds or fertilisers or foodstuffs. If the practical knowledge of the purchaser is not considered sufficient to enable him to purchase his seeds without the safeguard of an Act of Parliament ensuring their purity and their germination, or if the sale of fertilisers and feeding stuffs must similarly be safeguarded against the fraudulent selling of articles which do not contain the minimum of manurial constituents in the one case, nor the necessary food elements in the other, how can it be assumed that in the case of fungicides and insecticides sufficient knowledge exists with the grower, where an even greater acquaintance with scientific facts is necessary.

It has been said that the great trouble is, that legislation to deal with fungicides and insecticides whether secret or of a declared nature, must lead to dealing with proprietary medicines. There is no doubt whatever of the demand for legislation on the part of growers: through the National Farmers' Union, through the Chamber of Horticulture and through the Federation of British Growers, the demand is clear and repeated. The question is, is it better in the national interests to keep open the opportunity for clever advertisers to trade on the public ignorance and credulity to sell articles "worth a guinea" that cost a half-penny to produce, than to protect the growers of vital food from being led to waste their money and time on the use of articles, designed to destroy pests which cannot destroy them and which often injure the trees besides involving serious loss of crop from the very pests they were designed to destroy? Examples of the evil to be remedied lie about. A remedy

professing to kill Silver Leaf was tested for two years under an agreed scheme with practical and scientific assessors, but there was no agreement about the publication of the Report. The Report was not favourable and its publication is prohibited under pain of legal process for damages. A remedy purporting to cure Wart Disease of potatoes was promoted on the ground of a crop of potatoes on infected land being free of the disease. It was discovered that the potatoes chosen for trial were an immune variety.

The foundation principle of any Government action must be to enable the buyer to know what he is buying, as is done under the Fertilisers and Feeding Stuffs Act. To do this, there must be a system of Certificates or Licences, after due authoritative tests. These tests may be either practical in the field or chemical in the laboratory. The first class of tests will be expensive, must be prolonged and require much land. The second class can be conducted quickly and inexpensively, on the same principle as is done already with disinfectants.

Mr Theodore Parker said: One of the most difficult problems that I had to face when I entered the insecticide trade some years ago, was to realise that it was necessary to prostitute, to some extent, one's scientific ideals, to the requirements of the commercial side of the trade, because I had, and still have, although I must say in a lesser degree, a particular dislike to the sale of any commodity savouring of the patent medicine type of product.

Until that time I had never realised the great part that advertising played in the sale of insecticides, and I was of the opinion that the introduction of suitable legislation might eliminate the advertiser who offered specifics of the 101 cure-all type, and who made exorbitant claims, which it was very doubtful could be substantiated when put to the test.

An insecticide, to be of any use commercially, must be tried out on a large field scale, not in one district only, but in several, and under varying conditions of climate, soil, and last but not least, local *modus operandi*. The most striking characteristic of the horticultural trade, is that everybody seems to have different ways of doing things, and I very soon realised that the success or failure of an insecticide depended to a very large extent upon the man who applied it. I found that in the majority of cases, the application was left to the foreman, who might, or might not, have a working knowledge of the English Imperial measure. This generally resulted in the use of the product contrary to instructions, and in the addition of a little more concentrate to give the diluted wash "more body."

This state of affairs exists to a lesser extent to-day, but it means that the manufacturer has had to make his product fool-proof, in order to protect himself. In other words, the gross ignorance, and old-fashioned prejudice displayed by many of the growing fraternity in these matters, has, in my opinion, contributed largely to their general exploitation by the unscrupulous and unscientific business man.

The war gave an impetus to the use of chemicals for pest destruction, and with it the leading manufacturers made a really genuine attempt to put the insecticide trade on to a scientific basis.

Personally I should like to see insecticidal and fungicidal chemistry raised to such a status as exists in Canada and the States. Until the grower has been educated to the value of spraying etc. according to a more or less set programme compatible

with our varying climatic conditions, and the use of more or less standardised chemicals and formulae, he is likely to listen to the plausible story of the smart and efficient salesman.

I am afraid that the economic workers have sometimes been to blame, in that they have advocated methods of control which have not been altogether commercial propositions, and which have not appealed to the peculiar and particularly conservative mentality which characterises the grower.

During the past four or five years, it has been my privilege to lecture before the various Growers' Associations throughout this country and the Channel Islands, and I have been struck by the thirst for reliable information on the use of insecticides. It is certainly not the manufacturer's business to educate the grower, although I am afraid that a great deal is left to him. Post-war conditions have produced a class of grower which it is pleasant to find is gradually increasing. I refer to the younger generation who have had the opportunity of securing a fairly good grounding in scientific principles as applied to Agriculture and Horticulture.

The tendency with these growers is to purchase their chemical requirements on an analytical basis, and to some extent under a guarantee, hence there is a slowly increasing demand for high grade products, which must conform to certain specifications. This is a trend in the right direction and would, I believe, gradually starve out the manufacturers who rely mostly upon soap, water and smells as a basis for the active ingredients in their washes.

At present there are, I am afraid, a large percentage of growers who would find it difficult to interpret or to use the information which would of necessity be given on the label of an insecticide, where it was necessary to declare the percentage toxic ingredients.

There is undoubtedly room for improvement, and I for one would welcome any change which would raise the status of insecticidal and fungicidal chemistry. With a higher state of efficiency there would be I am sure, a more extended use of insecticides among the pessimistic and unbelieving, but there is another factor—What is one to do when a grower demands that his wash shall not cost more than $\frac{1}{2}d.$ to $1d.$ per gallon of diluted wash? His only alternative is to make it up himself, which believe me, under farming conditions, and with the unskilled labour available, is a most unsatisfactory process.

When a grower buys say a nicotine wash, he should be in a position to know if he is getting value for money. Even if he is, the results obtained after application may be anything but satisfactory, because he may not have had sufficient knowledge of the correct types of nozzle to use, the hardness of the water used, the desirability of using spreaders, or the compatibility of spray mixtures, which he may have mixed indiscriminately.

It appears to me, that if we are to make progress, there must be more mutual confidence between the manufacturers and the economic advisory officers. The plant pathologist must be the medical adviser, and the manufacturer's chief chemist the dispenser, watching both the interests of the grower and his employer by ensuring proper compounding and standardisation of products before despatch.

If the time does come which necessitates Government legislation in regard to insecticides, I personally should like to see some form of registration and approval,

such as exists for the sale of Sheep Dip, without the declaration of toxic ingredients, for the following reasons:

The leading insecticide firms in this country do maintain Research Departments which involve a monetary expenditure running into four or five figures in some cases, and often without achieving any commercial results.

It would be dangerous from the manufacturer's point of view and would not serve any useful purpose where a reputable firm was concerned, to broadcast generally the name of an entirely new insecticidal agent as active constituent in a newly marketed wash even if it had the highest possible scientific approval behind it, simply because it is not possible to protect or patent any chemical compound.

After expending time and energy, the producer is certainly entitled to protect, to some extent, the results of his research department's work, because there are so many people who would be ready to copy and imitate, and to utilise other people's brains, and to undercut in price to a considerable extent, particularly as their overhead charges are a negligible quantity. Of such people one can only say "they toil not neither do they spin."

A further objection to declaration of ingredients, is that it would tend to hamper and deter commercial enterprise and research, a most undesirable state of affairs.

My remarks concerning research work being carried out by insecticide firms may perhaps surprise some here to-day, and even raise a sceptical smile, but there are lines of research going on to my own knowledge, on a range of complex organic compounds, which may revolutionise pest control, and greatly add to our small number of recognised insecticidal agents.

To publish these without some form of protection would mean throwing away the results of many months' tedious and patient research work, at any rate from the commercial point of view.

Where there exists a special method of manufacture which is more or less a trade secret, then the declaration of the toxic ingredient does not matter.

There does not appear to be any difficulty in the way with regard to what are known as standard products, and with these there is a real need for improvement. For instance why lime sulphur solution is sold on a gravity of 1·300 is entirely beyond my ken. The mere fact that specific gravity is the standard specification encourages the unscrupulous to bring up the gravity to the standard by the addition of soluble salts. It has been shown by Salmon and Horton that all the constituents of a factory-boiled lime sulphur are inactive as fungicides with the exception of polysulphide sulphur. The determination of this constituent is quite simple from the analytical point of view, and the selling and buying on a guaranteed polysulphide sulphur content would surely be more satisfactory for everybody. The same remarks apply to liver of sulphur and ammonium polysulphide.

Arsenate of lead is more or less rationally standardised.

Bordeaux mixtures and indeed all copper fungicides should be sold on a guaranteed copper content, and there should be stated in addition, on the American lines, the amount of lime, *e.g.* 4.4.50 etc. which means 4 lb. sulphate of copper, 4 lb. of lime to be mixed with 50 gals. of water.

Flowers of sulphur for dusting purposes. I have had occasion to examine a large number of samples recently, and there appears to be a great difference in relative

sizes of the particles, and degrees of fineness of division, a sample recently examined being the best that I have so far handled.

It gave on sieving 70·0 per cent. passed a 200 mesh sieve.
„ „ 20·0 „ „ 150 „
„ „ 10·0 „ „ 120 „

In order that flowers of sulphur may be effective from the point of view of adhesion, and fungicidal value, at least 90 per cent. of a sample should pass the 200 mesh screen.

Soap. There are many qualities of soap offered for sale to-day containing varying percentages of moisture, from 25 to 60 per cent. The demand is for a soft soap made with potash, on account of its greater solubility in water than one made from soda. Soap certainly should be sold on a fatty acid content, it should also be stated if resin is used in the manufacture. In my opinion a soap made from a fish oil is certainly superior in killing properties to an ordinary vegetable oil soap. I know many growers who would disagree with me on this point, but I think it largely a question of preference and prejudice.

Dry dusts. We are passing through the transition period from wet spraying to dry dusting, but I am not going into the relative merits of either method of application.

The bases of most dusts are an inert carrying material, and nicotine. Now it appears to me that it should be insufficient to state merely the percentage of nicotine contained; what is of more importance, is the degree of fineness in state of subdivision of the carrier, together with the floating capacity and amount of nicotine retained by the carrier after it has been exposed to atmospheric conditions in a thin layer for so many hours. With some inert carriers it has been shown that only a proportion of the nicotine is liberated even after several hours have elapsed.

Emulsified oils. There has been a rather extended use during the past two years of this class of material as a dormant wash. Where claims are made that such washes possess egg killing properties the percentage of high boiling oils is insufficient; there should be some indication as to the nature of the oils, whether of aliphatic or aromatic origin. Another very important point, especially where hard water is concerned, is a declaration of the type of emulsification used in the manufacture.

It should be stated whether of a chemical or physical nature.

I have indicated a few points which would have to be considered in any scheme involving registration, or legislative control.

There are however one or two other instances which I should like to mention which indicate some of the difficulties that confront the insecticide manufacturer.

For example custom demands that a wash for commercial purposes be used at a dilution of 1 gallon to 80 to 100 of water; this means that 1 gallon of the concentrated material must contain sufficient of the active ingredient to kill at the prescribed dilution in addition to the soap necessary for purposes of spreading.

It is now recognised that for any soap wash to have good spreading properties at least 10 to 15 lb. per 100 gallons of water is desirable. It is obviously impossible to get such an amount into 1 gallon of finished product, consequently unsatisfactory results are recorded. This factor I think explains the apparent inefficiency of many products now offered for sale. The great cry of the growing fraternity is for cheaper insecticides, and in order to satisfy this demand the manufacturer naturally looks

for cheaper sources of supply of raw materials or available supplies of chemical by-products. While this is sound in many respects there is a danger of pit-falls.

When utilising or at any rate attempting to use waste products it is false policy to introduce these at the expense of insecticidal efficiency.

No washes to-day are super-efficient. Another matter of equal importance, which cannot be impressed too strongly on manufacturers, is that having produced a wash which has passed the stringent tests of both grower and research station, it is wise to leave well alone.

By this I mean that the trade generally has fallen into disrepute through good washes having been adulterated with cheap substitutes in an attempt to cheapen the cost to the grower.

In conclusion may I say one word about soil fumigants?

There are numerous specifics offered for sale under fancy names which claim to kill wireworm and kindred soil pests. The general composition of these it is not necessary to comment upon.

It is I think a usually accepted fact that there is no substance known which, if applied under commercial conditions, and as a commercial proposition, will kill or eradicate wireworms, yet such soil fumigants are offered, and large commercial growers buy considerable quantities year by year.

Why is this—do they act as deterrents? My own opinion is, that as most of them contain crude phenolic bodies, they act as mild sterilising agents to the soil, and so exert a slight stimulus to plant growth which may carry the plant over a critical stage at which it would have been attacked.

Fancy proprietary names I know are a bugbear, but they have their useful purpose.

As I have previously said one cannot register or protect a chemical compound, but by selling it under a proprietary name the manufacturer has a means of at least registering a name and to some slight degree protecting the product sold under its shelter, until the competitor's chemist obtains a sample for analysis, with the usual inevitable result.

Mr Tattersfield thought that while the interests of the horticulturist should be regarded as paramount in this matter of insecticides and fungicides, it was necessary to remember there were other interests involved and that to protect the horticulturist it was probable a rather elaborate machinery might have to be devised, in which both research worker and analytical chemist would have to play a part.

It was his opinion that any legislation or set of regulations on this matter should be so carefully devised as to cause a minimum of irritation to all parties concerned, otherwise a good deal of injury might be done to those interested in manufacturing insecticides and fungicides.

On abstract grounds it was perhaps debatable whether the fruit-growers had more right to Government protection than other business interests purchasing proprietary goods. But it should be borne in mind that the buying of insecticides and fungicides might demand very special skill and one could not expect the horticulturist to carry out elaborate tests on samples submitted to him.

Mr Tattersfield considered that some measure of publicity was necessary for the complete protection of the fruit-growers from fraud, and it was probable that

reputable manufacturers exaggerate the damage likely to be done to them by a declaration of contents. The research stations would explore this field more intensely in the future, and by the publication of results, render secrecy about the composition of insecticides and fungicides of less importance to the manufacturers.

For the successful working of any elaborate set of regulations some kind of machinery such as that which Mr Fryer had stated to exist in America would appear necessary and for its successful working some kind of declaration of contents would be desirable owing to the difficulty and labour involved in carrying out successful analyses of complex proprietary articles.

A declaration would act as a labour-saving device, for there was some risk of any authority, to which this work of testing was allocated, being inundated with samples, particularly if its imprimatur were placed on those passing its standard of efficiency. The closest collaboration of chemist, entomologist and mycologist would also be imperative for successful operation.

It would be necessary for any set of regulations to be sufficiently elastic to allow compounds to find their way readily into use, and if it was considered that a public declaration would do harm to enterprising manufacturers, it might be advisable to consider whether in certain cases a declaration made in confidence to the Government Department or Testing Authority might not be substituted.

Mr Petherbridge said: The question of "secret substances" has now become of marked economic importance to those who have to advise the actual growers. A few years ago advisers were able to recommend substances in the "declared" class equal to any in the "secret" class. That is not so to-day. A few years ago a Dutch firm introduced into this country a Carbolineum winter wash which proved effective for the purpose of killing the eggs of Aphides and Apple sucker. This is now the most widely used winter wash in my area. I now recommend proprietary articles as the most economic means of dealing with certain troubles with this proviso "that the article is satisfactory provided it is similar to that sold in certain years under the same name."

The difficulty of advisers is now considerably increased by the putting on the market by various horticultural chemists of a number of Carbolineum washes somewhat similar to the Dutch product. In order to advise growers as to whether a particular English "Carbolineum" is as good as the Dutch product I have to carry out a series of tests.

It would simplify matters very much from the advisers' standpoint if the Ministry were in a position to test these articles officially and to give an official report; but I realise that this would be a difficult and costly undertaking, and I think the necessary money could possibly be better employed for the growers' benefit in the carrying out of fundamental research on insecticides and fungicides.

The registration of "proprietary articles" stating within certain limits the composition or method of preparation would help to prevent a manufacturer from altering the composition of an article without changing its name.

It is very important that no action should be taken which is at all likely to hinder the activities of those manufacturers who are making an honest attempt to fulfil the wants of the growers.

Even with sprays of the "declared" class, many of which are very complicated

mixtures, no action should be taken until very careful research has been made into the toxic ingredient or ingredients, and then only after discussion with the manufacturers concerned.

Mr F. T. Brooks supported the view that legislative action should be taken to prevent the sale of fungicides and insecticides which were clearly of a fraudulent nature. He cited one example of a fungicide which according to the instructions of the manufacturers should be diluted at the time of application with *sterilised* water! He urged also that further facilities should be afforded to advisory officers and others who had to advise growers for testing the fungicides and insecticides of reputable firms, indicating that if this were done it might be necessary to obtain information as to the nature of these substances. He considered that legislation concerning fungicides and insecticides on reasonable lines such as those proposed by Mr Fryer would not lead to the stifling of initiative such as was feared by Professor Lefroy.

Mr Bunyard said: Considering the small amount of evidence before us as to damage or waste caused by using useless or deleterious spray fluids, I am of the opinion that the cost of setting up and maintaining machinery for carrying out the legislation proposed would far outweigh the amount of crop-loss sustained and is therefore not economical.

In my opinion, the loss of crops caused by improper application and mixing of *good* spray fluids is probably far greater than that sustained by using inferior preparations and for this reason I hold that propaganda by the Ministry would be far more beneficial than legislation.

The better application of good spray fluids would produce better results, thereby emphasising their superiority to the detriment of inferior compounds which would be gradually dropped in consequence.

Mr Goodwin said: The division of insecticides and fungicides into the two groups as suggested by Mr Fryer was convenient, for in the "declared" class where the active ingredient was known and could be determined by chemical analysis it was reasonable that the amount should be stated. Methods of analysis whilst not yet perfect were sufficiently reliable and capable of being carried out by commercial firms. In the absence of any statement or guarantee it was impossible for the user to judge of the value of a preparation or to check the price charged for it. Recognition must on the other hand be given to the fact that manufacturers did devote a considerable amount of time and money to the production of spraying materials of the "secret" class. It was hoped in this way to obtain an article which would, if satisfactory, become a monopoly and earn larger profits than could be obtained from standard articles. In such cases if the patent laws could not protect the makers and prevent the results of their work being appropriated, there should be some form of registration—something like the copyright of a book—with the Ministry of Agriculture or other central authority. The disclosure of the composition of such articles would of course be confidential and the registering authority would—once a register was compiled—be able to check to some extent the standard of the article as sold from time to time.

Dr Voelcker said: That, so far as the user—be he fruit-grower or horticulturist—was concerned, the desiderata in regard to insecticides, fungicides, etc., were: first, that they be efficient; second, that they be of regular composition. The pur-

chaser had a right to demand this, and legislation—just as in the case of the Fertilisers and Feeding Stuffs Act, of the benefits of which he had had much experience—might well be introduced to secure these ends.

Mr Fryer, in his opening, had very clearly and properly divided the articles to be dealt with into two classes, (*a*) the "declared," (*b*) the "secret," preparations.

In regard to the former, there was no reason why they should not be sold under a guarantee of being—within reasonable limits—of a certain nature and strength, and also as not containing anything that would be hurtful to vegetation, etc.

As to the second class—the "secret" preparations—he confessed that he felt some sympathy with the manufacturer who, after going to considerable expense and trouble, had "hit on" something that might be advantageously used, or on some particular way of preparing a substance, or of compounding certain materials in such form that they could be more readily applied—very naturally did not feel that he should be called on to make a full disclosure of the contents and of his methods of preparation, for the benefit of his trade rivals.

Just as in the case of a prepared food, or even an artificial manure, *everything* did not turn upon the analysis, and two men might make up one or the other, to contain the same percentage of valuable constituents, and yet, by reason of better condition, or more careful preparation, or, possibly, the addition of something that made the whole "work" better, there might be a not inconsiderable practical difference in favour of one as against the other. The man who had adopted the better method was certainly entitled to his reward.

Apart from this, however, he thought that legislation should be introduced to ensure that everything sold for a specific purpose should be able to satisfy the Authorities that it was effectual, and it was also necessary to secure that it be kept up to the declared nature and strength.

He quite approved the suggestion made by Mr Goodwin that there should be a "declaration in confidence," to the Authorities, of the composition of all "secret" preparations, and it was for the Authorities to put it to the test, and, when satisfied as to its efficacy, to grant a licence for its sale, just as had been done in the case of sheep dips, etc.

Mr E. S. Salmon congratulated Mr Fryer on the very lucid way in which he had treated a difficult subject, and expressed himself in full agreement with the views advanced. There were some cases, he considered, where a mycologist after carefully testing a patent preparation and finding it worthless, should take his courage into his hands and declare it so. One definite case might be mentioned where he had carried out experiments with a certain widely advertised and much used proprietary preparation sold to the farmer as a remedy against "Bunt" in wheat. The analysis of this substance, carried out by Dr W. Goodwin, showed that it contained approximately 5 per cent. copper sulphate and 81 per cent. iron sulphate, the remainder being water and insoluble matter. When diluted according to the instructions issued, there would be only 0·5 per cent. copper sulphate present. Now the experiments carried out at Wye College have shown that copper sulphate at 1 per cent. does not control "Bunt." Experiments using iron sulphate showed the uselessness of that substance for the purpose. It was not surprising therefore that when the preparation itself was used, in carefully controlled experiments, it was found to be useless for the control of "Bunt." It was intended to publish these results, naming the preparation and stating that when it was applied at such and

such a time and in such and such a manner, it proved, under the conditions of the experiment, useless for the control of "Bunt." He considered that further action on such lines required to be done.

Mr Salmon in commenting on some of the remarks made by Prof. Lefroy pointed out that if it were true that a worthless patent (or proprietary) insecticide died a natural death speedily, it was certainly not true of such fungicides. The instance given above was one such case. Moreover, it was perfectly clear, from the figures given by Mr Fryer of the great differences found in the composition of the various commercial preparations of lead arsenate on the market that legislation was urgently needed to standardise at least one valuable insecticide. This matter was of importance for mycologists also. In certain recent spraying experiments carried out on farms in Kent for the control of Apple scab, the mixture made of lime sulphur plus arsenate of lead had given very encouraging results. It became dangerous however to recommend farmers to use such a mixture, until the purchase of a legally guaranteed lead arsenate was possible.

Mr Fryer in his reply said: I am afraid that in the time available I cannot attempt to sum up the very interesting contributions which have been made to the discussion. I should like, however, to make it clear that I do not consider all secret preparations bad—the majority, I believe, are entirely good, but owing to the existence of a number of fraudulent articles, they have all got rather a bad name. I, personally, do not hanker after legislation for the secret type of insecticide or fungicide. If in the interests of farming or fruit-growing such legislation is necessary, then it must come, but the prospect for the unfortunate officials who have to administer the Act is anything but inviting.

I may also make it clear that I assumed from the title of the discussion that Government intervention was alone under consideration. There are other possibilities which must not be forgotten. When I was in Holland I was struck by the fact that the difficulties we have been discussing never seem to arise there and the only explanation I could find was that the agricultural and horticultural industries there are well organised in specialised groups, each with a strong association. Under such circumstances there would be little difficulty in the growers, through their association, dealing with fraudulent articles without any form of Government intervention. Or, again, if Government intervention is going to prove so destructive to initiative in the insecticide and fungicide industry, the firms themselves could go far to render such intervention unnecessary by themselves organising and warning the public against the article sailing under false colours. Doubtless at first they would have to fight libel actions, but they could afford to do so and the ultimate result would be practically assured. Acting, however, on my original assumption that our discussion is limited to Government intervention, I feel Dr Goodwin's solution is the right one—and that a revision of the Patent Law so as to give manufacturers real protection in the case of new discoveries for a period of five to seven years would remove practically all the objections to the declaration of active ingredients in the secret class of preparation. The chief difficulty in the way of legislation would thus be removed.

Finally, looking at the discussion from the point of view of an official of the Ministry of Agriculture, may I say how valuable has been the expression of views on the subject from such widely separated and diverse points of view.

REPORT OF THE COUNCIL FOR THE YEAR 1924.

During the year eight meetings have been held at which the average attendance has been some 45 members and visitors. The Council regrets that the attendance has been smaller than in recent years.

With one exception all the meetings have been held in London at the Imperial College of Science and Technology.

The Provincial meeting was held at Leeds University; there was an attendance of 86 including a fair number of members from London. The meeting on the first day was devoted to the reading of papers and a general discussion of the problem of the Propagation of Fruit Trees. On the second day a visit was made to farms in the neighbourhood where Rhubarb Culture and Diseases of Rhubarb were demonstrated. The meeting was a great success thanks to the admirable organisation of Professor Priestley and his colleagues.

During the year one honorary and seven ordinary members have been elected and seven members have resigned. The total membership excluding those whose subscriptions are three or more years in arrears now stands at 257 as against 243 last year.

The thanks of the Association are due to Prof. J. B. Farmer and his colleagues for their unfailing kindness and hospitality in granting the use of rooms for the meetings of the Association.

1924.

Jan. 18. Annual General Meeting:
Professor E. B. Poulton, Presidential Address: "The Relations of Pure and Applied Biology."

Feb. 22–23. Annual Provincial Meeting, at Leeds University:
Mr G. T. Spinks: "The Propagation of Fruit Trees on their own roots."
Dr R. C. Knight: "Experiments on the Rooting of Hardwood Cuttings."
Dr W. Robinson: "Vegetative Buds on Leaves of *Cardamine pratensis*."
Professor J. H. Priestley: "Vegetative Propagation of Flowering Plants."

Apr. 4. Sir John Russell: "Agricultural Conditions in the Sudan."

May 9. Mr F. T. Brooks: "Moulds attacking Meat in Cold Storage."
Dr Franklin Kidd: "Some Functional Diseases of Apples under Low Temperature Conditions."
Dr A. S. Horne: "Fungal Diseases of Apples kept in Cold Storage."
Dr D. Haynes: "Chemical Change in Stored Apples."

June 27. Visit to the Wembley Exhibition.

Oct. 24. Dr F. A. E. Crew: "Genetics and the Stock-breeder; an Account of the Work of a Research Institution."

Nov. 14. Discussion opened by Mr J. C. F. Fryer: "The General Principles that should underlie Government Action regarding Fungicides and Insecticides."

Dec. 12. Dr J. Munro: "The Control of Bark Beetles in Britain."
Mr B. P. Uvarov: "Recent Light on the Locust Problem."

REPORT OF THE HON. TREASURER.

The accounts for the year ending Dec. 31st, 1924 are shown on p. 304.

The expenses of the Association have been considerably in excess of those of the previous year mainly owing to an increase of £132. 8*s*. 10*d*. on the cost of the annual volume of the *Annals of Applied Biology*. This increase is due firstly to the greater size of the volume as compared with that of the previous year. Secondly, there has been a falling off in receipts derived from the sales of back volumes and reprints. It is satisfactory, however, to report that there has been no decrease in the number of trade subscriptions. Income from the latter source amounted to £337. 4*s*. as compared with £331. 12*s*. for the previous year. We have extended more generous treatment to authors of papers and only in one instance has a grant in aid of the cost of the publication of a paper been called for.

Our income from current subscriptions amounted to £258. 14*s*. as compared with £242. 16*s*. for the previous year—an increase of £15. 18*s*.

With regard to the balance sheet the excess of assets over liabilities appears large. This is made up of our investment of National Savings Certificates whose value is approximately £428. 18*s*. 3*d*. and the estimated value of the stock of our journal with the publishers amounts to £64. 2*s*. 6*d*. The actual uninvested cash balance to be carried over for 1925, after all charges have been met, amounts to £112. 4*s*. 2*d*. The general financial position of the Association may therefore be regarded as quite satisfactory.

A word may be added with regard to the investment in Savings Certificates. This sum includes the balance of money from the Publication Fund and benefits derived from increased sales of the *Annals* immediately following the war when, for the time being, the greater part of the income of the Association was not required to meet the cost of production of that journal. It is important that we should have assets of this character as the Association has on more than one previous occasion had to draw upon outside sources for financial assistance.

TREASURER'S STATEMENT FOR THE YEAR ENDING 31 DECEMBER, 1924

CASH ACCOUNT.

Cr	£	*s.*	*d.*	*Dr*	£	*s.*	*d.*
Jan. 1. Cash at Bank . . .	88	7	10	Postage	4	10	11
Dec. 31. Subscriptions:				Stationery and minor printing	5	7	0
A. Current . . .	258	14	0	Treasurer	172	18	4
B. Arrears . . .	44	9	0	Secretary	3	19	8
C. Advances . .	10	17	3	Balance at Bank	129	17	5
Entrance Fees . .	7	6	0	Placed on Deposit	100	0	0
Contribution to cost of papers in *Annals*	3	0	0				
Bank Interest . .	3	19	3				
Total	£416	13	4	Total	£416	13	4

BALANCE SHEET.

Liabilities.	£	*s.*	*d.*	Assets.	£	*s.*	*d.*
Subscriptions in advance . .	10	17	3	Current a/c	129	17	5
Liability on *Annals*, vol. XI .	303	1	0	Deposit a/c	250	0	0
Excess of assets over liabilities	604	18	11	Subscriptions two years or less in arrears and considered good	36	5	0
				Contribution owing on cost of paper in *Annals*, vol. XI . .	10	0	0
				Approximate value of 500 Savings	428	12	3
				Estimated value of stock of *Annals of Applied Biology* with publishers	64	2	6
Total	£918	17	2	Total	£918	17	2

A. D. IMMS, *Treasurer.*

We have examined the Treasurer's statement of expenditure and receipts and have found it correct. We consider that the above balance sheet correctly represents the position of the Association. — J. C. F. FRYER. R. STENTON.

REVIEW.

The Erythrocyte and the Action of Simple Haemolysins. By Eric Ponder. (Biological Monographs and Manuals, pp. x + 192. Edinburgh and London: Oliver and Boyd. 1924. 12*s.* 6*d.* net.)

This volume is the second of the series of biological monographs and manuals now being issued under the editorship of F. A. E. Crew and D. W. Cutler, and, if the later volumes are of the same excellence as this, the series should be useful as well as interesting. The red cell is so easily isolated and so easily observed and offers apparently such excellent material for the study of many cellular characters, such as structure, composition, permeability and so forth, that the literature is enormous; and it is convenient to have so much of it so lucidly summarised and so critically handled as we have it here. The author's self-confident dogmatism is uniformly irritating, but perhaps this need not be wholly regretted, since it has the stimulating effect of other irritants; but it inevitably raises doubts whether previous workers have been so consistently unenlightened and their labour so valueless as is here represented and whether if Mr Ponder were to attack his own work with the same severity he might not modify some of his conclusions. The book falls into two parts, the first dealing with the morphology, chemistry and structure of the erythrocyte, and the second with the phenomena attending, and the mechanism underlying, the action of simple lysins (as distinct from such complex action as serum haemolysis). In the former, besides the main discussion on the mammalian red cell, there is a useful compendium of the present information on the cells of fish, birds, reptiles, amphibia (in which Amphioxus is included!) and invertebrates; in the latter the action of hypotonic saline, heat, saponin, bile-salts, acid and alkali, and ultra-violet rays, these lysins being chosen as representatives of groups of similar action. Finally, there is a chapter on the mechanism underlying the lytic process, and a discussion on the data supplied by curves showing the progress of haemolysis with lapse of time and curves showing the effect of dilution of the lysin on the time required to produce the action. This follows the lines now usual, and is clearly expressed. There is no statement of the effect produced on the shape of the percentage-haemolysis curve (and consequently on the derived resistance-frequency curve) by altering the strength of the lysin, though there is reason to suppose that this effect is considerable and it leads to difficulties in the resistance-interpretation, which Mr Ponder does not touch upon. On the whole an interesting and provocative book.

J. HENDERSON SMITH.